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VOLUME 34 SEPTEMBER 1956 NUMBER 5

INTERACTION OF TEMPERATURE AND LIGHT ON THE SEEDLING REACTION OF McMURACHY WHEAT TO RACE 15B OF STEM RUST[1]

BY F. R. FORSYTH[2]

Abstract

The reactions of seedlings of McMurachy wheat to stem rust (*Puccinia graminis* Pers. f. sp. *tritici* Erikss. & Henn.) race 15B were determined in growth chambers under various combinations of temperature and light. Experiments in continuous light on seedlings kept at temperatures alternating between 62° F. and 72° F. showed that the critical period determining reaction to rust was the time at which symptoms of infection (i.e. flecking) became visible. Experiments involving temperatures of 60°, 70°, and 75° F. at a photoperiod of 16 hr., with 8 hr. of darkness, showed that a 16-hr. period of low temperature (60° F.) and light induced resistance to the rust if this period fell between 51 and 115 hr. after inoculation but was most effective if it occurred between 75 and 91 hr.

Introduction

The reaction of many cereal varieties to physiologic races of rust is markedly influenced by temperature. Temperature was considered to be the most important factor in the breakdown of seedling resistance in the studies by Waterhouse (7) on stem rust of wheat and oats, Johnson (5) on stem rust of wheat, Gordon (3) on stem rust of oats, Peturson (6) on crown rust of oats, and Gassner and Straib (2) on leaf rust of wheat. Carefully controlled studies on the reaction of seedling wheat plants to stem rust, in which both temperature and light were considered as interacting factors, have not been reported.

The wheat varieties Selkirk and McMurachy are known to be resistant to race 15B and most other stem-rust races at low temperature (60° F.) but become susceptible to race 15B and some other races at constant temperatures of 75° F. or above (4). In the field, plants of these varieties remain resistant even when temperatures are considerably above 80° F. in the early part of the afternoon whereas it was observed in the present study that seedling plants grown in growth chambers at 72° F., with a photoperiod of 16 hr. followed by 8 hr. of darkness, were susceptible. These facts suggested that an interaction of temperature with some other environmental factor might influence the host–parasite relationship.

[1]*Manuscript received May 2, 1956.*

Contribution No. 1534 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.

[2]*Plant Physiologist, Plant Pathology Laboratory, Winnipeg, Manitoba.*

[The July number of this Journal (Can. J. Botany, 34 : 419-743. 1956) was issued August 2, 1956.]

This paper describes the relative effects of temperature and light on the stem-rust reaction of McMurachy seedlings grown under controlled conditions.

Materials and Methods

Seed of McMurachy wheat was planted in soil in 3½ in. pots and germinated in chambers until the seedlings were ready for inoculation at the two-leaf stage. Uredospores of race 15B of stem rust were used to inoculate the first leaves of five or six plants in each pot. The plants were incubated at 70° F. in the dark for 24 hr.

One set of experiments (Table I) was conducted with continuous light and variations of temperature. In the other set (Fig. 1) a photoperiod of 16 hr. followed by 8 hr. of darkness was combined with different temperature periods. Continuous records of temperature and humidity were kept. The temperature fluctuated ±1° F. and the relative humidity varied about 8% in the chambers. Lighting was entirely by fluorescent lamps and at the plant level, in these experiments, the light intensity was about 450 foot-candles.

Experimental Results

Table I summarizes experiments with plants kept in continuous light. Plants kept at a constant temperature of 72° F. were susceptible (Sample 1). If transferred from 62° F. to 72° F. one day before flecking (three days after inoculation) they were also susceptible (Sample 2). If transferred at approximately the time of flecking (four to five days after inoculation) or at any

TABLE I

THE REACTIONS TO RACE 15B OF WHEAT STEM RUST OF SEEDLINGS OF SELKIRK AND MCMURACHY KEPT FOR DIFFERENT PERIODS, FOLLOWING INOCULATION, AT 62° F. AND 72° F. IN CONTINUOUS LIGHT

Sample	Days kept at each temperature after inoculation			Rust reaction*
	72° F.	62° F.	72° F.	
1	2	0	9	S
2	2	1	8	MS
3	2	2	7	MR
4	2	3	6	MR
5	2	4	5	R
6	2	5	4	R
7	2	6	3	R
8	2	9	0	R
9	3	8	0	MR
10	4	7	0	MS
11	5	6	0	MS
12	6	5	0	S
13	7	4	0	S
14	8	3	0	S

= *Resistant.*
MR = *Moderately resistant.*
MS = *Moderately susceptible.*
= *Susceptible*

subsequent time, they were resistant (Samples 3 to 9). Once flecking had commenced, the warm period could not overcome the effect of the cold.

If the seedlings were transferred from the original incubation temperature of 72° F. to 62° F. even just before flecking and were maintained at the lower temperature they showed a susceptible reaction (Samples 10 to 14). Once flecking had commenced, the cold period could not overcome the effects of the initial warm period.

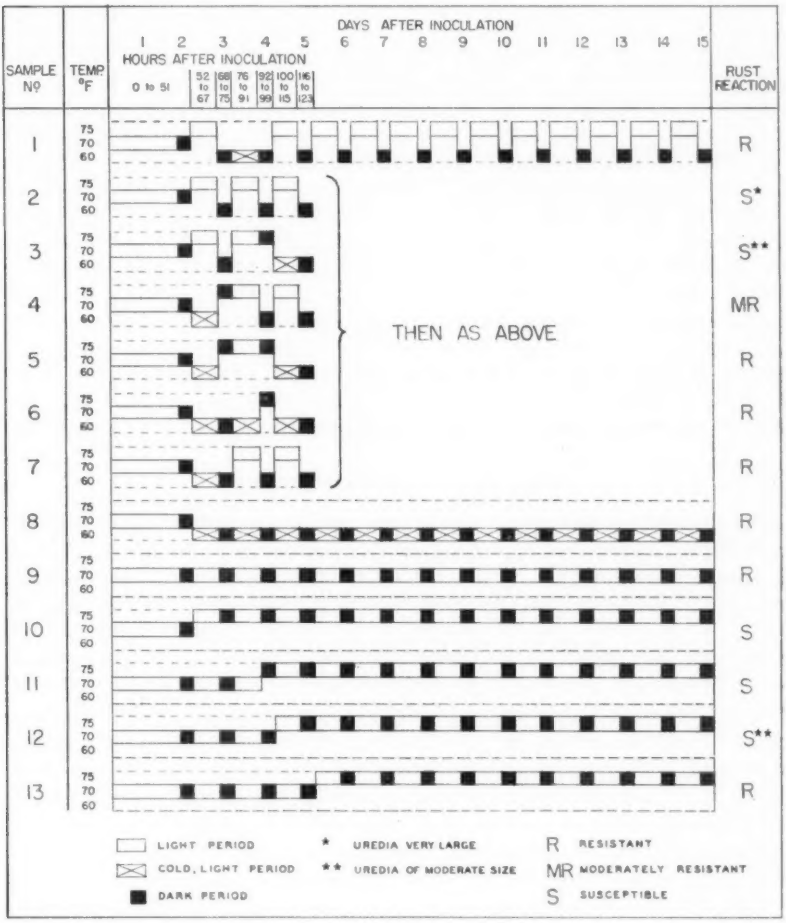

FIG. 1. Samples 1–7.—Effect of one to three critical, cold, light periods on the reaction of McMurachy seedlings to race 15B of stem rust.

Samples 8, 9, 10.—Effect of continuous 60°, 70°, and 75° F.

Samples 11, 12, 13.—Effect of increasing the length of time that the seedlings are maintained at 70° F. before transfer to 75° F.

In another experiment in continuous light it was found that a 16-hr. period at 72° F. alternating with an 8-hr. period at 60° F. resulted in a resistant reaction to race 15B of stem rust in McMurachy wheat. But if the plants were kept four days or longer at a steady 72° F. before the alternating temperature program commenced, the seedlings were susceptible.

Fig. 1 summarizes the experiments with alternating light and dark periods combined with alternating warm and cold periods. A susceptible reaction is obtained with alternating warm and cold periods (75° and 60° F.) provided that the cold periods are dark (see Sample 2). However, one to three 16-hr. periods in the cold and light, before the 120th hr. after inoculation, influence the reaction towards the resistant type. This influence is first shown in Sample 3 by a decrease in pustule size. It is apparent from Samples 4 and 5 that the resistance increases with increasing number of cold, light periods. Furthermore, from a comparison of Samples 1, 3, and 4 it is obvious that the cold, light period between 75 and 91 hr. after inoculation is the most effective of the three periods under consideration: 51 to 67, 75 to 91, and 99 to 115 hr. Sample 4 in which the cold, light period is followed by a warm period in the dark shows a slight increase in susceptibility compared to Sample 7 in which the cold, light period is followed by a cold, dark period. Samples 8, 9, and 10 show that resistance results at steady temperatures of 60° or 70° F. respectively and susceptibility results at a steady temperature of 75° F. A comparison of Sample 13 with 11 and 12 indicates that a steady temperature of 70° F. results in a resistant host reaction which is not reversible after the 123rd hr. but which is fully reversible at the 91st and partially reversible at the 99th hr.

Discussion and Conclusions

The 16-hr. period of low temperature and light, used in these tests, is considerably longer than the cold, light periods found in nature during the months of June and July in Manitoba. At that time of year these periods extend from daybreak to the time the temperature rises to 74°–75° F. Towards the end of June, 1955, and in the first half of July, those periods averaged seven hours in length. In the latter half of July there were on the average only four hours of light with low temperatures (60°–75° F.) in the morning. Results from studies with inoculated seedlings grown in the growth chambers indicate that the effects of the cool periods are cumulative. Thus one or two 16-hr. cold, light periods might be expected to have about the same effect as two or four 7-hr. cold, light periods. However, the present study does not prove that the effect of the 7-hr. cold, light periods in nature is the principal environmental factor influencing resistance in Selkirk and McMurachy, though the evidence suggests very strongly that such is the case.

Forward (1) found that the subjection of seedlings of 11 wheat varieties, inoculated with race 21 of wheat stem rust, to darkness for periods of from one to seven days resulted in the modification of the infection type towards resistance. The metabolism of leaves grown in the dark and of leaves grown in the light at low temperatures may have some common basis which modifies

the host–parasite relationship towards resistance. Possibly other combinations of wheat variety and rust race, which are not thought to be sensitive to temperature, will be affected by a sufficiently long, cold, light period before flecking. The explanation of the causes of the resistance shown by seedlings grown in the dark and those grown in cold and light is not known in terms of shifts in metabolic patterns.

Green and Johnson (4) concluded that in adult wheat plants inoculated with various races of stem rust "high temperature apparently did not affect the host alone because no variety showed increased susceptibility to all races, nor was the effect produced on the rust alone because no race showed increased virulence on all varieties". It has been established by the present work that there is a relatively short period falling about three to four days after penetration of the rust into the host, during which the host–parasite relationship is so labile that light and temperature conditions can shift this relationship one way or another. There is no knowledge at present on the mechanism of this action but possibly the initial effect is primarily on the host. In any event, the ultimate effect of low temperature in the presence of light during this period is the establishment of a resistant host reaction.

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THE NUTRITION OF PHYTOPHTHORA[1]

By L. E. Lopatecki[2] and William Newton[3]

Abstract

Phytophthora cactorum grew well in ammonium–salt media but not in nitrate. Conversely, *P. megasperma* grew well in nitrate but not in ammonium–salt media. *P. parasitica* and *P. erythroseptica* grew well in both, but better in that containing nitrate. All species grew better in the presence of organic nitrogen. Evidence is presented that iron, zinc, copper, and manganese are essential trace metals and that zinc is required in greater concentrations than iron. The optimum requirement for glucose or sucrose was found to be approximately 4%, and the residual sucrose was converted into reducing sugars by all species except *P. cactorum*. Approximately 50% of the residual sugar remained unchanged in the cultures of this species. The growth rate and sugar consumption increased as the thiamine level was increased from 0.2 to 0.8 mgm. per liter, but the coefficient of sugar utilization remained constant. On the other hand, the percentage of nitrogen in the mycelium decreased markedly as the thiamine content of the medium was increased. It is concluded that with media containing 1% alanine an accurate bio-assay of thiamine can be made over a concentration range of 0.2 to 0.6 mgm. per liter.

Introduction

Phytophthora species were notoriously difficult to cultivate on synthetic media until thiamine was proved to be an essential nutrient (4, 7, 9). It is interesting that this appears to be the only vitamin required. Most species appear to be able to utilize inorganic nitrogen salts but their growth rates are invariably increased when the nitrogen is supplied in organic forms (5, 12). The macro nutrient requirements of four species of *Phytophthora* are reflected by the present study, together with the influence of trace elements, thiamine, and sugar upon *P. parasitica*, information which may be of value in the utilization of this species in the bio-assay of thiamine.

Materials and Methods

The *Phytophthora* species used in these experiments were *P. cactorum* isolated from an apple crown-rot lesion, *P. parasitica* from tomato-fruit lesions *P. erythroseptica* from potato tubers (pink rot), and *P. megasperma* from lesions on the roots of spinach.

The fungi were cultured in 50-ml. flasks containing 20 ml. nutrient solution and incubated for 21 days at 20° C. The mycelial mats were washed with 100 ml. distilled water, dried at 90° C., and weighed. The mean weight of the mats of quadruplicate cultures were recorded as the measure of growth.

Residual sugars were determined by a modification of the Shaffer–Hartmann method (11), residual alanine by the Van Slyke manometric procedure, and total nitrogen by the Kjeldahl method using a selenium catalyst.

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Contribution No. 318 from the Chemistry Division and No. 1537 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.
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TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS, GROWTH THEREIN, AND pH AT THE END OF THE INCUBATION PERIOD

Solutions	1	2	3	4	5	6	7	8	9
Composition, gm./liter									
Dextrose	50	50	50	50	50	50	50	50	50
KH_2PO_4	1	1	1	1	1	1	1	1	1
Thiamine	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
KNO_3	4	4	4	4	—	—	—	—	—
$MgHPO_4$	—	0.1	—	—	—	—	—	—	—
$MgSO_4$	—	—	0.1	0.1	0.1	0.1	0.1	0.1	0.1
$CaSO_4$	—	—	—	0.1	0.1	0.1	0.1	0.1	0.1
$(NH_4)_2HPO_4$	—	—	—	—	4	—	—	—	—
Asparagine	—	—	—	—	—	4	4	4	—
dl-Alanine	—	—	—	—	—	—	—	—	4
$FeSO_4$	—	—	—	—	—	—	0.001	0.001	0.001
Trace minerals*	—	—	—	—	—	—	—	1 ml.	1 ml.
Growth, mgm.									
P. parasitica	Nil	52	63	112	49	118	210	245	186
P. cactorum	Nil	4	7	2	35	46	111	150	146
P. megasperma	Nil	1	10	65	5	113	178	269	239
P. erythroseptica	Nil	67	73	115	39	131	210	233	175
pH									
P. parasitica	—	5.6	5.8	5.6	3.2	6.1	6.0	5.9	5.9
P. cactorum	—	4.7	4.8	3.7	3.3	6.0	6.1	6.1	6.0
P. megasperma	—	4.2	4.3	4.6	3.6	5.9	5.8	6.0	6.0
P. erythroseptica	—	5.4	5.4	5.6	2.8	6.2	5.8	6.2	6.2

**1 ml. of the trace-mineral solution adds 1 p.p.m. zinc and 0.02 p.p.m. molybdenum, copper, and manganese.*

In the trace-element study, a special effort was made to avoid contamination. All glassware was soaked overnight in 10% nitric acid, rinsed thoroughly with distilled water, and finally autoclaved while full of glass-distilled water. Steinberg's procedure (10) was used to purify the basic constituents of the media. This involved the autoclaving of concentrated solutions of the media with calcium carbonate and decanting the supernatant portions for use. Asparagine and *dl*-alanine were purified by repeated precipitation from ethyl alcohol.

The sugars were autoclaved separately from other constituents of the media and added when cold. This was done because growth seemed to be retarded when dextrose was autoclaved with other constituents, particularly asparagine and *dl*-alanine.

Experimental Procedure

Gross Nutrient Requirements

The composition of the nine incomplete and complete nutrient solutions is shown in Table I together with records of growth in each by four species of *Phytophthora*, and the pH of the solutions at the end of the incubation period. The records of growth show that in the inorganic nitrogen cultures *P. cactorum* grew well when supported by an ammonium salt (solution 5) and poorly in the presence of a nitrate salt (solution 4). Conversely, *P. megasperma* grew well in nitrate and poorly in the ammonium–salt cultures. *P. parasitica* and *P. erythroseptica* grew well in both, but better in the nitrate cultures. The better growth of all species in solutions 6, 7, 8, and 9 than in 4 and 5 suggests that all *Phytophthora* species are better supported by organic than by inorganic nitrogen. It may be noted from the data in Table I that slightly better growth was obtained when supported by asparagine than by equivalent amounts by weight of *dl*-alanine. This has been studied further and the results will be reported in a later paper.

The Metabolism of Glucose and Sucrose

The data on the metabolism of glucose and sucrose in the media are shown in Table II. It is apparent that both sugars are equally effective as carbon sources and that the optimum concentration is close to 4%. *P. cactorum* appeared to be slightly more tolerant of sucrose concentrations above 4%. A probable explanation was discovered by examining the nature of the residual sugar, that which remained in the sucrose cultures at the end of the incubation period. All the residual sucrose had been converted to reducing sugars except in the *P. cactorum* cultures where approximately 50% remained as sucrose. It would follow that the rise in osmotic pressure through the hydrolysis of the sucrose would be less rapid in the *P. cactorum* cultures than in the cultures of the other species, which may account for the less rapid depression of growth.

TABLE II

THE METABOLISM OF GLUCOSE AND DEXTROSE

Species	Initial concentration sugar, %	Glucose		Sucrose	
		Mycelium, mgm.	Residual sugar, %	Mycelium, mgm.	Residual sugar, %
P. parasitica	2.0	132	Nil	130	Nil
	4.0	183	1.5	189	1.4
	7.0	160	4.4	172	5.2
	10.0	141	8.0	164	7.7
P. cactorum	2.0	120	Nil	122	Nil
	4.0	166	1.5	168	1.8
	7.0	144	4.7	154	4.1
	10.0	134	7.3	123	7.5
P. megasperma	2.0	118	Nil	120	Nil
	4.0	244	Nil	245	Nil
	7.0	202	3.7	238	3.7
	10.0	198	6.2	187	6.5
P. erythroseptica	2.0	147	Nil	118	Nil
	4.0	175	1.4	180	1.5
	7.0	170	4.5	187	5.0
	10.0	160	8.1	171	8.0

Trace Mineral Requirements

The results of a study of the trace mineral requirements of *P. parasitica* are shown in Table III. Solution 8 (Table I) purified by the Steinberg procedure was used. Manganese, copper, and molybdenum were added to give concentrations of 0.05 p.p.m. and zinc and iron to give 1 p.p.m., and these trace minerals were omitted to obtain proof of their essential nature. In one series of cultures, tap water was added to the complete mineral solution. It may be noted that the data support a conclusion that iron, zinc, copper, and manganese are essential, and that molybdenum is either unessential or the purification of the ingredients of the medium has left enough of it to support growth, and that an unknown essential substance appears to be present in local tap water.

TABLE III

GROWTH OF *P. parasitica* ON A PURIFIED BASIC MEDIUM AS INFLUENCED BY TRACE MINERALS AND TAP WATER (QUINTUPLICATE CULTURES)

Additions	Relative growth
None	20
All minerals	87
Minerals and tap water	100
Minerals less iron	50
Minerals less zinc	30
Minerals less copper	74
Minerals less manganese	60
Minerals less molybdenum	85*

**Difference insignificant between all minerals and minerals less molybdenum.*

TABLE IV

GROWTH OF *P. parasitica* AS INFLUENCED BY IRON AND ZINC CONCENTRATIONS (QUINTUPLICATE CULTURES)

Iron	Relative growth	Zinc	Relative growth
None	60	None	45
0.1 p.p.m.	89	1.0 p.p.m.	91
1.0 p.p.m.	100	2.0 p.p.m.	98
2.0 p.p.m.	78	5.0 p.p.m.	100
100.0 p.p.m.	76	100.0 p.p.m.	97

The results of an attempt to determine the optimum requirements of iron and zinc are shown in Table IV. The data suggest that zinc is required in larger amounts than iron and that iron but not zinc depresses growth at concentrations of 2 p.p.m. or higher. The trace minerals had not been completely removed from the basic medium by the $CaCO_3$ purification treatment, as indicated by the dithizone test (1). The substitution of asparagine by *dl*-alanine or glycine in the basic medium did not effect a better removal; the explanation is, undoubtedly, because these organic nitrogen compounds hold metals tenaciously by chelation (2).

The Influence of Thiamine upon Glucose and dl-Alanine Metabolism

The influence of thiamine concentration upon the metabolism of glucose by *P. parasitica* is shown by the data in Table V. The optimum concentration of thiamine appears to be around 0.8 mgm. per liter in solution 9 (a complete nutrient that contained 4 gm. *dl*-alanine per liter) as measured by the amount of sugar utilized by the fungus or by the dry weight of the mycelial mats produced in the cultures. The coefficients of utilization (the weights of the sugar utilized divided by the weights of mycelial mats) are constant at thiamine levels of 0.2 to 0.8 mgm. per liter. On the other hand, the percentage of nitrogen in the mycelial mats decreases as the thiamine content of the nutrient solution and the rate of sugar utilization by the fungus increases.

TABLE V

THE INFLUENCE OF THIAMINE CONCENTRATION UPON THE COEFFICIENT OF UTILIZATION OF GLUCOSE AND UPON THE NITROGEN CONTENT OF THE MYCELIA OF *P. parasitica*

Thiamine, mgm./liter	Mycelia, mgm.	Glucose utilized, mgm.	Coefficient of utilization	Nitrogen content of mycelia, %
0.2	68	185	0.38	4.38
0.4	137	348	0.39	3.32
0.6	195	530	0.37	2.95
0.8	197	531	0.37	2.80

Growth Curves as Influenced by Thiamine and dl-Alanine

The influence of thiamine and *dl*-alanine at various levels is shown in Fig. 1. The curves indicate that when the media contain 1% alanine, the growth response to thiamine is linear between concentrations of 0.2 and 0.6 mgm. per liter, and therefore within this range *P. parasitica* could be used in the bio-assay of thiamine.

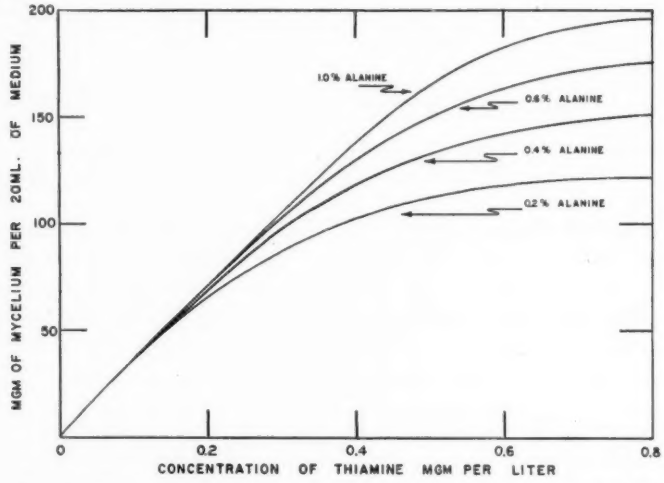

FIG. 1. The growth of *P. parasitica* as influenced by *dl*-alanine and thiamine.

Discussion

It is apparent from this study that distinct species of *Phytophthora* differ markedly in their ability to utilize ammonium and nitrate salts. The capacity of *P. cactorum* to utilize the ammonium and its comparative inability to utilize nitrate has been noted also by Hawker (3) but no explanation is offered. We also are unable to explain why *P. megasperma* can utilize nitrate and has little ability to utilize the ammonium ion. An explanation might be found through an intimate study of the influence of the H ion owing to its rapid accumulation through the metabolism of ammonium salts, but insufficient pH data were taken in this study to draw any deductions.

The evidence that iron, manganese, copper, and zinc are required in the nutrition of *P. parasitica* is not contrary to expectations, but the evidence presented of higher zinc than iron requirements is perhaps surprising. Owing to our inability to remove traces of metals from the basic nutrient solution, it cannot be said that we have obtained positive proof that molybdenum is not essential. An unknown essential substance is present in local tap water and is probably a trace mineral but attempts to identify the substance have failed.

The possibility of utilizing *P. parasitica* as a test organism in the bio-assay of thiamine is clearly illustrated by Fig. 1. The chief advantage of using this *Phytophthora* species lies in the simplicity of its nutrient requirements. It appears that thiamine is the only vitamin required and that neither the pyrimidine nor the thiazole moieties of thiamine are utilized by the fungus for growth (5, 6, 8).

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THE NONUTILIZATION OF *d*-ALANINE AND GROWTH STIMULATION BY *l*-ALANINE IN THE NUTRITION OF PHYTOPHTHORA PARASITICA[1]

By William Newton[2]

Abstract

Barely significant growth has been obtained in cultures of *Phytophthora parasitica* containing *d*-alanine as the sole source of nitrogen, but abundant growth in those containing *l*-alanine. Growth was found to increase as the level of *l*-alanine was elevated from 0.2 to 0.8%, although 0.2% represents a greater quantity than is removed by the fungus. In experiments the growth of *Verticillium albo-atrum* was shown to be supported by *d*-alanine, although much less efficiently than by the laevo form.

Introduction

A previous study (1) of the utilization of organic nitrogen by *Phytophthora parasitica* indicated that better growth is obtained in a nutrient solution containing 0.4 gm. of asparagine per liter than in a solution containing an equivalent amount by weight of *dl*-alanine as the sole source of nitrogen. This study was conducted to learn the cause of the poorer growth in the alanine cultures. It appeared possible that the poorer growth was due to the exhaustion of nitrogen in the alanine cultures.

Method

Complete nutrient solutions were used in accordance with the methods that have been described (1). These solutions contained thiamine and trace minerals and were identical except with respect to nitrogen. This element consisted of alanine only in its several forms and at various concentrations. The amino acid that remained in the cultures at the end of the incubation period was determined by the van Slyke manometric procedure. The *dl*-alanine, the *d*-alanine, and the *l*-alanine, as received from the Nutritional Biochemicals Corporation, were used without additional purification.

Experimental Procedure

The growth response to different quantities of *dl*-alanine is shown by the data in Table I, together with the residual alanine at the end of a 21-day incubation period at 20° C. The growth data represent the dry weight of the mycelial mats produced in the cultures.

The data in Table I show that as the concentration of *dl*-alanine is increased from 0.2 to 1%, the growth of the fungus increases in spite of the fact that residual alanine is present at the end of the incubation period, even at the

[1]*Manuscript received May 4, 1956.*
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TABLE I

GROWTH OF *P. parasitica* IN RELATION TO THE INITIAL AND FINAL CONCENTRATIONS OF *dl*-ALANINE IN THE CULTURES (QUADRUPLICATE)

Initial concentration, %	Growth, mgm.	Final concentration, %
0.2	125	0.08
0.5	151	0.37
0.7	175	0.54
1.0	186	0.83
LSD 1%	6	0.05

lowest initial concentration. It is evident that this amino acid acts as a growth stimulant as well as a nutritive substance owing to the amounts that remain in the solutions at the end of the incubation period. It may be noted that approximately 50% of the alanine is not utilized in the cultures of the lowest initial concentration. This evidence suggests that one component only of the optical isomers of alanine is utilized.

The data in Table II show that *d*-alanine is not utilized by *P. parasitica*, or only to a slight extent. The slight but significant growth in the *d*-alanine cultures may be accounted for by the introduction of traces of supporting nitrogen with the inoculum or by impurities in the *d*-alanine. On the other hand, growth is well supported by *l*-alanine, and like the growth in the *dl*-alanine cultures, it increases as the concentration is elevated up to 0.8%.

Data upon the growth of *Verticillium albo-atrum* are included. The trials were conducted merely to learn whether other plant pathogens could utilize *d*-alanine. It may be noted that this fungus is supported by *d*-alanine, but not nearly so well as by the laevo form.

TABLE II

INFLUENCE OF *l*-ALANINE AND *d*-ALANINE UPON THE GROWTH OF *P. parasitica* AND *Verticillium albo-atrum* CULTURES (QUADRUPLICATE)

Initial concentration *l*-alanine, %	Growth, mgm.	Initial concentration *d*-alanine, %	Growth, mgm.
P. parasitica			
0.2	63	0.2	2
0.4	72	0.4	3
0.8	99	0.8	3
LSD 1%	4		0.07
V. albo-atrum			
0.4	212	0.4	82
LSD 1%	12		4

Discussion

The evidence that the growth of *P. parasitica* is supported by *l*-alanine but not by *d*-alanine suggests that studies of the metabolism of optical isomers and isomeric groups within organic compounds might lead to the creation of better nutrient solutions for the growth of fungi in liquid cultures. The catalytic or growth-stimulating effect of excess alanine in the nutrient solutions is difficult to explain. The degree of stimulation by the excess alanine appears to be greater than can be explained by the simple assumption that its rate of absorption by the fungus is stepped upwards by the mass effect. The stimulating influence of the excess alanine proved to be of the same order when the experiment was duplicated, except that the cultures were placed in a shaker. A pronounced rise in the growth rate was the only effect of the agitation. In 'shake' cultures, the growth of organisms is seldom diminished by lowering the concentration of nutrients providing they are in excess of requirements. Although there appears to be little previous work upon the metabolism of the optical isomers of alanine, the stimulation of germination and respiration of *Bacillus megaterium* spores by *l*-alanine but not by *d*-alanine has been reported recently (2).

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RAGWEED IN SOUTHEASTERN ALBERTA[1]

By E. H. Moss[2]

Abstract

Recent surveys show that common ragweed, *Ambrosia artemisiifolia* var. *elatior*, is prevalent in two regions of southeastern Alberta and adjacent Saskatchewan, (*a*) an area with Medicine Hat as approximate center, (*b*) a narrow area east and west of Empress. The weed appears to be firmly established in depressions and on slough borders of natural prairie and of cultivated land. Available evidence points to the conclusion that the species was introduced to the Canadian plains, though the possibility that it was indigenous there cannot be dismissed. The question of its probable spread westward and northward is discussed.

Introduction

During a study of air-borne pollen in Alberta, large amounts of ragweed (Ambrosiaceae) pollen were caught at the Medicine Hat station (4). Field work done at that time revealed considerable common ragweed, *Ambrosia artemisiifolia* L. var. *elatior* (L.) Desc., near Medicine Hat and also near Empress. These results indicated that further information on the incidence of ragweed in southeastern Alberta and adjacent Saskatchewan was desirable. Accordingly, during small portions of three summers, 1953 to 1955, surveys were conducted to discover the geographical occurrence of the weed in the general region and also to gain a better understanding of its ecological relationships on the Canadian plains.

Observations

Geographical Occurrence

The accompanying map (Fig. 1) shows most of the areas where ragweed was found. In the immediate vicinity of Medicine Hat, also near Empress, several additional stations were located but these are omitted from the map to avoid congestion. At almost every station there was a heavy infestation of the weed; the few areas with light infestations (scattered plants) were usually near the fringes of the main ragweed regions.

Two major ragweed regions, and one minor one, are indicated.

(*a*) A major region, centering on Medicine Hat, with an arm extending west to the Bow River near Ronalane. The most westerly station, a short distance west of the Bow River, is at the edge of the new Hays irrigation project. The heaviest infestation is north of Medicine Hat, extending a short distance into the Suffield Experimental Area.

(*b*) A second major region with Empress as the approximate center, being in the form of a rather narrow strip mostly on the south side of the Red Deer River from Buffalo to Empress in Alberta, and from Empress on the south side of the South Saskatchewan River almost to Leader in Saskatchewan.

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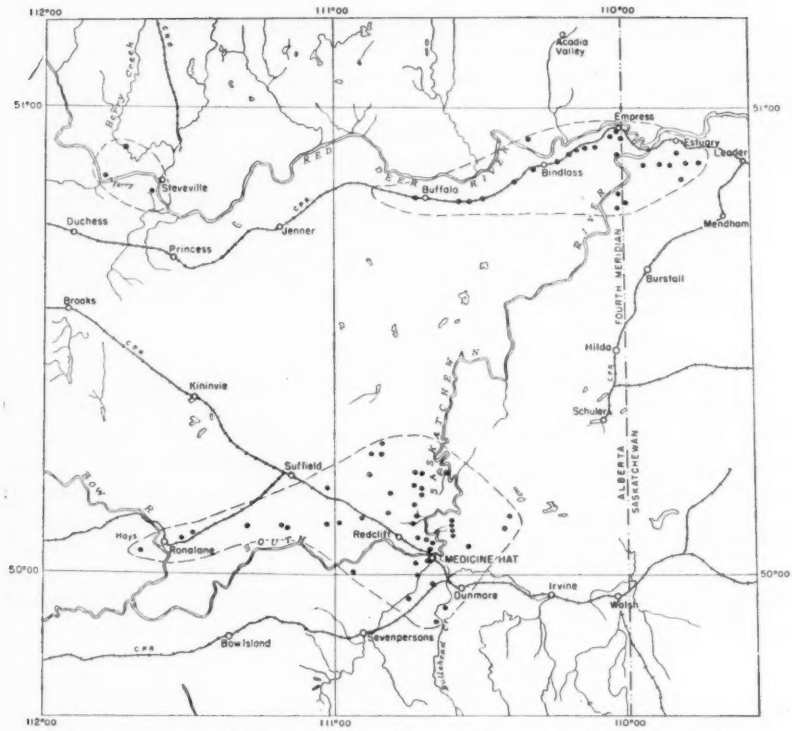

FIG. 1. Areas of ragweed infestation in southeastern Alberta and adjoining Saskatchewan.

(*c*) A minor region, with only three stations, north and northeast of Brooks, on or near the Red Deer River. This region may possibly be connected, by sporadic occurrences of the weed, with the Empress region of infestation. Other light infestations of ragweed might be expected farther west along the river, though none was found at Dorothy. It is significant that a thorough search revealed no ragweed in the irrigation areas of Brooks and Bassano. Nor was the weed found eastward from Medicine Hat in the region of Irvine and Walsh, though it has been reported (2) still farther east at Maple Creek, also at Swift Current, in Saskatchewan.

These regions are delimited with considerable confidence because extensive exploration of many contiguous areas failed to show any ragweed. Apparently the two major regions of infestation (Medicine Hat and Empress) are quite separate. The broad intervening zone of Schuler, Hilda, and Burstall, where there are numerous suitable sites for ragweed, was thoroughly explored with only negative results.

It may be added that investigations by the writer in many other parts of southern and southeastern Alberta, including Lethbridge – Taber – Vauxhall,

Foremost – Orion, Acadia Valley – Oyen – Alsask, Consort – Provost – Hardistry, and Drumheller – Hanna areas, have revealed no common ragweed. Actually, the weed has been reported for a very few stations within certain of these areas; but all available information points to the conclusion that these reports pertain to only sporadic occurrences of the plant. A sizable ragweed infestation, known for some years at Edmonton, south-central Alberta, is discussed below.

Habitats of Ragweed in the Region

The plant was most commonly found on the bottoms of depressions and on margins of ponds and small lakes. It occupied these habitats both in cultivated areas and in natural prairie. The occurrence of ragweed, along with native species, in depressions of unbroken prairie, sometimes several miles from the nearest cultivated fields, came as a distinct surprise. Ragweed was seen also on coulee bottoms and stream flats, and locally along roadside ditches. Only occasionally was the weed found in stands of grain. Even where wheat or oats grew close to a slough fringed with ragweed, there was usually an abrupt transition from the latter to the grain and rarely any of the weed in the grain. Only under the exceptionally good moisture conditions that had prevailed in the Empress–Leader region during 1954–55 was ragweed extending somewhat into grain stands from adjoining depressions.

Our observations indicate that ragweed tends to become established and to persist in depressions of the region. Moisture conditions in these places appear generally to be suitable for the weed and competition with other weeds and with native vegetation is often met successfully. There is some evidence that the plant is favored by occasional cultivation of slough borders and bottoms; but often it was found in dense stands or intermixed with other species in the apparent absence of artificial disturbance. Native species commonly associated with ragweed in these habitats include *Hordeum jubatum*, *Potentilla norvegica*, *Plagiobothrys scopulorum*, *Mentha arvensis*, *Eleocharis palustris*, *E. acicularis*, *Calamagrostis* spp., *Erigeron canadensis*, *Polygonum* spp., *Carex* spp., *Beckmannia syzigachne*, *Alopecurus aequalis*, *Limosella aquatica*, *Epilobium adenocaulon*, *Artemisia biennis*, and *Grindelia squarrosa* var. *quasiperennis*.

Discussion

Introduction of the Weed

When common ragweed first appeared in southeastern Alberta is difficult, if not impossible, to determine. Almost certainly it was introduced several decades ago. The present distribution pattern (Fig. 1) indicates two major centers of introduction and expansion, viz. Medicine Hat and Empress. If the weed has actually spread from these centers to its present coverage, the extension could hardly have occurred in less than a few decades. However, the possibility of a much shorter time cannot be dismissed. Further observations on the behavior of the weed at the fringes of its present areas may indicate the normal rate at which it establishes in new sites.

A further consideration, brought to the writer's attention by Dr. C. Frankton (3), is the possibility that common ragweed is native to the Canadian prairie region. It is generally granted that the species is indigenous to the Great Plains. Possibly it occurred in southeastern Alberta before settlement. An early collection (1879) of the species by Macoun in Saskatchewan, critically studied by Frankton as to date and locale, might be taken to establish that it is native to the Canadian prairies. However, even as early as 1879 the weed might conceivably have been brought to the region by travellers. Moreover, the present distribution pattern of ragweed in southeastern Alberta and the evident success of the weed in prairie depressions under natural conditions indicate that it was introduced. If native to the Canadian plains, it would surely have achieved a much wider distribution there than it has at present.

Extension and Control

In the light of the foregoing considerations, it may safely be predicted that common ragweed will continue to spread in southeastern Alberta and adjoining Saskatchewan. The two major areas of infestation are likely to enlarge, though the rate of expansion is problematical. Almost all parts of the country offer low moist sites, such as slough margins, for the establishment of the weed. The irrigation areas of Hays, Tilley, Brooks, and Bassano, to the west of the Medicine Hat region, might conceivably be invaded. It appears that agriculturists in Alberta and Saskatchewan should come to recognize common ragweed as a potential menace, with a view to practical control measures designed to prevent its spread from the present infested regions.

The question of a northward extension of common ragweed is an interesting one. At least some spread northward from the Empress–Leader region is a distinct possibility. Whether the species would be successful in the parkland and northern forest zones is problematical. Moisture conditions northward would presumably be even more favorable for the weed than in the Medicine Hat and Empress regions. On the other hand, the lush grass–sedge–willow vegetation of more northern depressions would probably militate against the success of ragweed in those sites. Moreover, the growing season northward is said to be unsuitable for production of seed by ragweed. According to Allard (1) this short day species in higher latitudes cannot fruit successfully owing to lower mean temperature and a shorter frost-free season. In eastern Canada, also in Europe, ragweeds are said to be poorly adapted to the prevailing cool climatic conditions north of latitude 50°. When introduced there, they often fail to persist for more than a single season. However, in Alberta, there is evidence that common ragweed can persist as far north as latitude 53° 30′. At Edmonton a patch of the weed along a railroad has been well established for several years. Evidently viable seed has been produced there, at least occasionally. It appears therefore that common ragweed may come to fruition at relatively high latitudes in Alberta. This behavior may prove to be of some importance for control of the weed in western Canada.

Acknowledgments

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CELL SIZE AND SURVIVAL IN CONIFER CAMBIUM[1]

By M. W. Bannan[2] and Isabel L. Bayly[3]

Abstract

The pseudotransverse divisions of fusiform initials by which the cambium of conifers is accommodated to increasing girth tend to occur at relatively high rates of frequency. The overproduction of new initials is accompanied by extensive cell loss. The continuation or reduction of fusiform initials after origin in anticlinal division is evidently related to cell length and extent of ray contacts. The largest fusiform initials generally survive and repeat the cycle of elongation and multiplication by pseudotransverse division. The cells of intermediate length continue or fail in varying proportions, the rate of failure tending to rise with increasing frequency of anticlinal division. As a rule the initials with the largest area of ray contacts persist and those with sparse ray contacts decline. The shortest fusiform initials are usually lost, passing off into maturation or undergoing further diminution to ray initials. The continued selection of the longest of the newly formed fusiform initials, which is operative at all stages in tree growth, undoubtedly contributes to the maintenance of an efficient cell length in the secondary vascular tissues.

Introduction

More than eighty years ago Sanio (23) made a survey of tracheid length in different parts of the tree, using a Scotch pine (*Pinus sylvestris* L.) for study. He arrived at certain conclusions one of which was that cell length increased outward from the pith, through a number of annual rings, until a definite size was attained after which there was no further elongation. This trend has been confirmed for other species of conifers by numerous investigators (1, 3, 4, 9–17, 19–22) but with the amendment that fluctuation in cell length may occur in the peripheral growth of mature trees.

Diameter growth and circumferential expansion entail multiplication of fusiform initials in the cambium. In the conifers this is brought about by pseudotransverse division of the initials (2, 18). It has been demonstrated (5, 8, 24) that these anticlinal divisions occur at a relatively high rate of frequency so that a potential overproduction of new cambial cells results. An intracambial competition for survival ensues and some cells are favored over others. In the present paper it will be shown that cell size and extent of ray contacts are important factors in survival. The continued selection of the longer initials obviously has a bearing on the tendency for cell length to increase outward from the pith.

Materials and Methods

The method of studying the sequence of changes in the cambium has been described in previous papers (5, 24). Owing to the fact that both secondary xylem and phloem replicate the cell pattern in the cambium, either of these

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tissue systems can be used for determining the history of changes in the meristem. However, because the limits of annual increments are more easily recognized in the xylem, that tissue was preferred. In the case of the local species trees of varied size were selected, samples taken from the peripheral wood at the 3–4 ft. level, and tangential sections cut in series to an aggregate thickness of 0.5–1.0 cm. From intensive study of the sections, the frequency of pseudotransverse divisions in the cambium was worked out, cell size determined before and after anticlinal division, and behavior of the fusiform initials recorded. It should perhaps be noted that the data on cell length presented on the following pages were derived from the xylem and hence are in error to the extent of elongation during periclinal division of the xylem mother cells and subsequent maturation. This amounts to 5–10% of the cell length, but since the error is more or less constant it has no bearing on the general conclusions.

Cell Length and Survival

As is well known, the multiplication of fusiform initials in the cambium of conifers takes place by pseudotransverse division. An inclined wall is laid down in cytokinesis so that the two daughter initials are generally somewhat longer than one-half the length of the original cell. Events after the division vary. Sometimes both daughter initials continue the repetitive cycle of enlargement and multiplication by anticlinal division. In other cases one of the daughter initials survives and elongates while the other becomes diminished, undergoing contraction and maturation to a permanent element or subdivision

TABLE I

RELATION BETWEEN LENGTH OF FUSIFORM INITIALS AT PSEUDOTRANSVERSE DIVISION AND BEHAVIOR OF DERIVED DAUGHTER INITIALS

Species	No. of trees	Stem diam. in ft.	Ring width in mm.	Mean length of fusiform initials at pseudotransverse division in mm.		
				Both derived initials survive	One derived initial fails	Both derived initials fail
Thuja occidentalis	1	0.3	3.1	2.62	2.30	2.24
Thuja occidentalis	15	0.8–1.3	0.5–1	3.25	2.93	2.63
Thuja occidentalis	19	0.8–1.3	1–3	3.06	2.83	2.48
Thuja occidentalis	8	0.8–1.3	3–4.5	2.72	2.46	2.25
T. plicata	2	3	0.8–2.0	2.97	2.63	2.19
Chamaecyparis nootkatensis	1	2	0.14	3.42	3.03	2.60
Cupressus arizonica	1	2	0.8	2.73	2.38	2.30
Heyderia decurrens	1	5	0.6	5.33	4.56	3.78
Sequoia sempervirens	3	5–6	0.3–0.9	7.10	6.47	5.06
S. gigantea	1	15	1.2	5.14	4.02	3.50
Abies concolor	1	3	1.0	7.15	5.73	4.20
Cedrus deodora	1	1.3	0.7	4.40	3.60	2.90
Tsuga canadensis	1	1	1.9	4.12	3.80	3.07
Pseudotsuga taxifolia	1	7	1.7	6.50	5.47	5.12
Pinus resinosa	1	0.1	4.1	2.01	1.89	1.63
Pinus resinosa	1	0.5	3.0	3.76	3.34	2.55
Mean ratio	—	—	—	1.37	1.19	1.00

to ray initials (7). Less often both daughter initials fail. A study of numerous sequences has revealed a relationship between the nature of events after pseudotransverse division and the length of the initial at the time of its division.

Data on the relationship between cell length and survival rate, derived from study of nearly 2400 anticlinal divisions, are presented in Table I. Here is shown the mean length of the original fusiform initials just prior to pseudotransverse division for three categories of postdivision behavior. It will be noted that the longer the fusiform initial at the time of its anticlinal division, the greater the probability of survival of both daughter initials. Decrease in length of initials at pseudotransverse division is accompanied by a rise in mortality rate of the derived initials. The positive relationship between cell size and survival was observed in all species studied. The correlation held in both slow growing and vigorous trees (*Thuja occidentalis*). It was also maintained at all stages in tree growth, from youth (*Thuja occidentalis*) to great age (*Pseudotsuga* and *Sequoia*).

It should be emphasized that the data in Table I pertain only to pseudotransverse divisions in apparently normal fusiform initials, that is, to potentially additive divisions. From time to time fusiform initials are lost from the cambium, and this failure is usually accompanied by contraction in tangential width, loss in length, and often by subdivision to short cells some of which may continue as ray initials. No measurements were made of cells which had begun to contract before pseudotransverse division.

Further confirmation of the bearing of cell size on survival is revealed in analysis of the data relating to the daughter fusiform initials recently formed in anticlinal division. For this purpose, *Thuja occidentalis*, the most studied species, will be used. A grouping of the newly formed daughter initials into three size classes discloses that among the upper one-fourth, comprising the longest cells, 90% survived and only 10% were lost from the cambium. By way of contrast, in the lower fourth, consisting of the shortest cells, 12% of the daughter initials continued and 88% failed. In the middle half, made up of cells of intermediate length, the proportion of survivors ranged from 38–85% in different specimens.

It is clear that the process of pseudotransverse division by which the cambium of conifers accommodates itself to increasing girth is accompanied by a continued selection of the largest initials. The survival of the longest cells and elimination of the shortest is obviously a factor in the maintenance of an adequate cell length, and doubtless contributes in some measure to the increase in mean cell length which takes place during the growth of the first several years. More important elements in the latter trend are, of course, a falling off in the frequency of anticlinal division during the early years and a delay in the incidence of multiplicative division relative to cell length. Fluctuations in mean tracheid length in the peripheral increments of mature trees, such as have been noted by several investigators, appear to be due largely to variations in the frequency of pseudotransverse division. The rate of

TABLE II

LENGTH AND RAY CONTACTS OF SURVIVING AND FAILING DAUGHTER FUSIFORM INITIALS SHORTLY AFTER ORIGIN IN PSEUDOTRANSVERSE DIVISION

Species	No. of trees	Stem diam. in ft.	Ring width in mm.	Surviving fusiform initials			Failing fusiform initials		
				Length in mm.	No. of contact ray cells	No. of ray cells per mm. of length	Length in mm.	No. of contact ray cells	No. of ray cells per mm. of length
Thuja occidentalis	9	0.8–1.3	0.5–1	1.89	16.8	8.9	1.60	10.3	6.4
Thuja occidentalis	10	0.8–1.3	1–3	1.85	19.7	10.6	1.54	12.6	8.2
Thuja occidentalis	6	0.8–1.3	3–4.5	1.60	19.8	12.4	1.33	12.2	9.2
T. plicata	2	3	0.8–2.0	1.81	27.2	15.0	1.43	15.4	10.8
Chamaecyparis nootkatensis	1	2	0.14	1.96	23.9	12.2	1.47	13.3	9.1
C. lawsoniana	2	1.2–1.3	1–3	1.90	22.0	11.6	1.56	14.6	9.4
Cupressus arizonica	1	2	0.8	1.60	23.3	14.6	1.32	15.0	11.4
Heyderia decurrens	1	5	0.6	3.00	44.5	14.8	2.27	26.7	11.8
Sequoia sempervirens	3	5–6	0.3–0.9	4.14	42.9	10.4	3.26	26.9	8.3
S. gigantea	1	15	1.2	2.88	45.1	15.7	2.02	25.7	12.7
Abies concolor	1	3	1.0	3.75	97.5	26.0	2.66	53.0	19.9
Cedrus deodara	1	1.3	0.7	2.38	44.5	18.7	1.79	21.3	11.9
Tsuga canadensis	1	1	1.9	2.45	41.4	16.9	1.97	25.2	12.8
Pseudotsuga taxifolia	1	7	1.7	3.65	65.6	18.0	2.91	44.1	15.2
Average of means	—	—	—	2.49	38.1	14.7	1.94	22.6	11.2

anticlinal division in late growth varies widely. A range from 0.1 to 4.8 successive pseudotransverse divisions per centimeter of xylem increment was observed in the peripheral growth of *Pseudotsuga* and *Sequoia* trees varying from 5–15 ft. in diameter.

The Ray Contacts of Fusiform Initials

In an earlier paper (6) it was pointed out that the area of contact with rays is highly important in the survival of fusiform initials. Data on the relationship between cell length, extent of ray contact, and behavior are presented in Table II. The statistics on length and number of bordering ray cells are mean values and they pertain to daughter fusiform initials at the time of their inception, that is, as observed shortly after origin in pseudotransverse division. The extent of ray contact generally alters later as a consequence of cell growth, loss of neighboring fusiform initials, or intrusion of the tips of nearby elongating cells. However, since the survival or early failure of newly formed fusiform initials is related to the conditions which obtain at the time of origin, all measurements of length and counts of adjoining ray cells were made at that early stage. The data refer not only to case histories in which the two daughter initials resulting from anticlinal division were of contrasting behavior, one continuing and the other failing, but also to other series in which both daughters survived or both were lost. The data were compiled from study of 3100 cells.

Similar relationships between length of the newly formed fusiform initials, extent of ray contact, and behavior were noted in all species. The fusiform initials which continued were slightly longer on the average than those which were lost, and they had contacts with nearly 70% more ray cells. A comparison on the basis of ratio of ray cells to length of initial shows that the surviving initials had contact with 30% more ray cells per millimeter of length than had the failing cells. Similar differences are revealed when the comparison is restricted to those series with contrasting cell behavior, one daughter fusiform initial continuing and the other failing (Table III).

TABLE III

LENGTH AND RAY CONTACTS OF NEWLY FORMED FUSIFORM INITIALS IN *Thuja occidentalis*

Source of data	Surviving fusiform initials			Failing fusiform initials		
	Length in mm.	No. of contact ray cells	No. ray cells per mm. of length	Length in mm.	No. of contact ray cells	No. ray cells per mm. of length
All series	1.81	18.6	10.3	1.52	11.7	7.7
Contrasting series, one daughter initial surviving and the other failing	1.70	18.2	10.7	1.58	11.9	7.5

As noted earlier, the data in Tables II and III are given in the form of mean values. There is, of course, much variation from cell to cell. Occasionally long cambial cells fail and relatively short cells survive. The range of variation is indicated by the following statistics. In one sampling of *Thuja occidentalis* the surviving daughter fusiform initials were 1.75 ± 0.28 mm. long and had contacts with 16.0 ± 5.9 ray cells. The dying fusiform initials were 1.44 ± 0.32 mm. long and bordered 9.8 ± 5.3 ray cells. The magnitude of the standard deviation reflects considerable variation.

The importance of ray contacts may be demonstrated by another analysis of the data. In *Thuja occidentalis* a comparison of the remaining daughter fusiform initials which were under mean length (for the survivors) with the declining initials which were above mean length (for the failures) showed the latter to be 12% longer than the former, but the slightly shorter continuing initials were in contact with 45% more ray cells per millimeter of cell length. In these borderline cases extent of ray contact was clearly an important element in the survival or failure of the fusiform initials.

Discussion

The rate of pseudotransverse division in the cambium of conifers fluctuates widely but is usually such as to produce a greater number of initials than required in circumferential expansion. The nature of the stimulus causing anticlinal division is undetermined, but it is clear that the rate of division is not geared to increase in girth. A consequence of the overproduction of new fusiform initials is an intracambial competition for survival and selection of cells of certain preferred types. Usually the longest cells continue and the shortest fail. The selection of the longer cells, which is operative at all stages in tree growth, is highly important in the maintenance of an effective cell length. This is especially true when the rate of anticlinal division is accelerated, as is sometimes the case in the late growth of mature trees. A continued high level of anticlinal division, without some compensating mechanism, would result in a drastic shortening of the vascular elements. The potentially detrimental effects of frequent pseudotransverse divisions are overcome by selection of the longest cells and rapid elongation of the newly formed fusiform initials between successive anticlinal divisions.

It has been shown that among the fusiform initials newly produced in pseudotransverse division the longest usually continue to grow and the shortest deteriorate in vigor and activity. In the middle half, consisting of cells of intermediate length, some continue and others are reduced, the ratio varying greatly in different samplings. In these cells the area of contact with rays seems highly important, the survivors generally having more extensive ray contacts than the failures, both in an absolute sense and proportionately to cell length. Evidently the rays are the source of substances required in the sustenance, growth, and division of fusiform initials. Carbohydrates doubtless rank high among these materials, being needed both for wall construction and the maintenance of turgor. It is significant in this

connection that the failing cells undergo tangential contraction indicating reduction in internal pressure. However, the existence of variable behavior, as demonstrated in the loss of some long fusiform initials with abundant ray contacts and the sporadic survival of short initials, points to a complex intercellular relationship.

The question arises as to whether length is a significant factor in the survival of fusiform initials apart from the ray contacts. It is noteworthy that when one pseudotransverse division is quickly followed by another so that a quartering of the mother cell results, or when the division is excentric so that one daughter initial is decidedly shorter than the other, the much abbreviated cells seldom continue as fusiform initials. The period of functioning may vary with the extent of the ray contacts, but the final results are similar. When the much shortened fusiform initials have sparse contacts with rays, they usually pass quickly into maturation or reduction to ray initials. If they adjoin large rays they may continue through the greater part of the growing season only to fail in the late summer. Since exceedingly few of the shortest fusiform initials are enabled to go through the usual cycle of enlargement and multiplication, even when well provided with ray contacts, it would appear that the element of size itself is important in survival.

In conclusion, it may be stated that the fusiform initials newly produced in pseudotransverse division are divisible into three categories on the basis of size and behavior. The longest usually continue and repeat the cycle of enlargement and multiplication by anticlinal division. The cells of intermediate length survive or fail in varying proportions, the survival rate tending to decrease as the frequency of anticlinal division rises. Comparison of the continuing initials in this group with those which fail indicates that the extent of ray contacts plays an important role in survival. No doubt this factor is also involved in the very high survival rate of the longest initials. These cells, because of their length, are usually well provided with ray contacts. The very short fusiform initials, on the other hand, almost always fail, even when bordering large rays. In the smallest fusiform initials an irreversible chain of events is apparently set in motion, and the consequence is maturation to reduced, malformed, permanent elements or continued diminution to ray initials. The later phases of this transformation are accompanied by a loss of longitudinal polar characteristics. It is noteworthy that whereas fusiform initials are cut down to smaller units some of which function as ray initials, the latter do not, except under unusual circumstances, expand to fusiform initials in conifer cambium.

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CHEMICAL AND BIOCHEMICAL RESPONSES OF THE MATURE SUGAR BEET ROOT TO DEFOLIATION BY FROST AND KNIFE[1]

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Abstract

The tops of mature field-grown sugar beets were frozen or removed by knife and the leaves allowed to regrow, or removed by knife and all regrowth continuously removed, for a period of 16 days. Sample roots were dug from the ground one, four, eight, 12, and 16 days after the original defoliation, and dry weight, sucrose, invert sugar, starch–dextrin, total nitrogen, and insoluble nitrogen determined. The activity of catalase, phosphorylase, phosphatase, *beta*-amylase, and invertase was measured. Compared with untreated plants, all treatments affected in a similar way the substances or activities measured, with the exception of phosphatase and catalase activity. Dry weight decreased, and percentage sucrose based on fresh weight, fell steadily, and was 2.89 below that of controls on the 16th day. On a dry weight basis the sucrose percentage of roots of defoliated plants did not differ significantly from that of roots of untreated plants. It is suggested that the continued fall in sucrose percentage on a fresh weight basis is the result of continued absorption of soil water. Invert sugar was higher in treated plants and increased with time. Changes in starch–dextrin content were small. The total N content of the various plants showed no significant differences. Insoluble N was not altered in a recognizable pattern. The activity of catalase and phosphorylase was lower in treated plants, while phosphatase and invertase showed increased activity. *Beta*-amylase activity was low in all roots.

Southern Alberta represents the northern fringe of areas suitable for sugar beet growth in North America and in this area fall frosts usually occur during the period of harvesting of the beets. Peto (4) made a study of the sucrose content of beets, used by the Canadian Sugar Factory at Raymond, Alta., for the 20-year period 1932–1951, in relation to the first killing frost. Subsequent to this frost, regardless of its intensity, there was usually a decreased percentage sucrose which was apparent within five days. This decrease reached a maximum in 1950, and the figure for sucrose percentage dropped 1.0 in five days and 1.1 in 10 days after the frost. Continued cold weather after the killing frost tended to prevent a loss and the occurrence of warm weather after the frost caused an increase in sugar loss. This loss was further emphasized by the fact that the normal rapid increase in sugar, which occurs at this time of year, was stopped at the time of freezing.

In small-scale experiments, Peto (5, 6) determined that removal of tops by frost or by knife resulted in comparable sucrose losses. Removal of the leaves and their subsequent regrowth was considered the primary cause of the sucrose loss. Data supporting the conclusion that sucrose loss may occur in the field following a heavy frost without leaf regrowth were presented and the opinion expressed that heavy frosts that penetrate the crown may be

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capable of reducing the sucrose content even though continued low temperatures prevent top regeneration. Wood (9) reported a sharp drop in sucrose content following a frost of −15° F. at Longmont, Colo., in 1951. The drop was not associated with new foliar growth.

In 1946, Price and Fife (7) found that sugar beets which had been topped but not lifted showed a gradual and significant decrease in percentage dry matter. This decrease they ascribed to absorption of water from the soil after the tops had been removed. Sucrose decreased significantly 10 days after topping. There was a gradual increase in reducing sugar in experimental beets, with a sharp increase occurring at the 10th day.

The investigation described below, supported by the extramural research grant E M R-50 of Science Service, Department of Agriculture, was designed to discover if possible whether there is an actual sucrose loss following removal of the tops of mature sugar beets by frost or by knife and followed by top regrowth in one instance and its prevention in another; what other chemical changes accompany the sucrose loss; and by what metabolic pathway the loss occurs. A study of the respiration of the sugar beet root and the effect of removal of tops on the pattern of respiration is being investigated at the present time.

Experimental

The preliminary work of 1953 involved sugar beet plants grown in pots of composted soil in the greenhouses of the University of British Columbia at Vancouver. The experiments of 1954 and of 1955 utilized field-grown beets. Since the organization and results of the two years' work were similar in most respects, the 1955 investigation will be discussed. Any important differences will be noted in the text.

Sugar beet seed, S.K.E.-R-11, obtained from the British Columbia Sugar Refining Co., Vancouver, B.C., was sown in flats in the greenhouse on Jan. 26, 1955. The seedlings were subsequently thinned, transplanted to other flats, and uniform seedlings planted in the field Mar. 26. The young plants were spaced 12 in. apart in 15 rows 30 ft. long and three feet apart. The soil was sandy loam and quite uniform throughout the plot. An application of well-rotted compost had been applied earlier in March. A side dressing of 10–20–10 fertilizer was applied immediately after planting and again in May and in July. The plot was watered by a sprinkler system. When treated and harvested on and after Sept. 1 the beets were vigorous in their growth, with roots six or more inches across. A number of the roots were somewhat globoid, rather than long and tapering. The average weight of the topped roots was 1279 gm. (2 lb. 13 oz.).

Four treatments, each applied to 18 randomized groups of six beets in the rows on Sept. 1, 1955, were as follows: (1) control, (2) tops frozen and allowed to regrow, (3) tops removed by knife and allowed to regrow, (4) tops removed by knife and all regrowth removed every second day. Freezing was accomplished by covering the groups of six beets with bottomless, insulated plywood

boxes 22 in. wide, 24 in. high, and 6 ft. long (Fig. 1), similar to that used by Peto *et al.* (6). On a wire mesh tray located in the upper part of the box were placed four 20-lb. blocks of dry ice (solid carbon dioxide). A one-hour treatment froze the leaves solid to within about an inch of the crown. No crown damage was observed. The leaves removed by knife were cut as close to the crown as possible. Measurements of temperatures within the box during the freezing period gave readings of −16° C., two inches above the ground, and −11° C., just below the dry ice.

One, four, eight, 12, and 16 days after defoliation on Sept. 1, one beet was pulled from each of five groups of similarly treated beets in a predetermined sequence, and the four most uniform of these used for analysis. Three such sets of four beets were analyzed separately at each time of harvest, hence 15 of the 18 repetitions of treatment were sampled at each of the five harvest dates. The beets were trimmed of leaves and small roots, cleaned, weighed, and the crowns removed. The entire remaining portion was sliced and put through a food chopper. The pulp, including the small amount of juice produced by pulping, was mixed for five minutes. Two 50-gm. aliquots were used for dry weight determinations at 85° C., two 26-gm. aliquots were transferred to Kohlrausch flasks for sucrose and invert sugar measurements, two 40-gm. aliquots were placed in a Waring blendor with 100 ml. ice water, blended for two minutes, strained through broadcloth, and the filtrate used immediately for enzyme activity determination. Three hundred grams was heated at 110° C. for 10 min. to stop enzymatic changes, dried at 85° C.,

FIG. 1. Freezing box. The boxes, which had no bottom, were placed over the beets, blocks of dry ice placed on the wire mesh tray, and the cover set in place. In the 1955 boxes, rock wool batts completely lined the removable lids.

ground to pass 100 mesh, redried, and stored over calcium chloride in desiccators. This powder was used for nitrogen and starch–dextrin measurements. All measurements were in duplicate.

Analytical Methods

Sucrose was determined by the standard method of the Association of Official Agricultural Chemists (1). Invert sugar was measured by pipetting two 5-ml. aliquots from the Kohlrausch flasks and determining the reducing sugars by the Somogyi – Shaffer – Hartman method (8). Total nitrogen including nitrates, insoluble nitrogen, and starch–dextrin were measured by methods described by Loomis and Shull (2).

Catalase activity in 3 ml. of filtrate, phosphatase, phosphorylase, and *beta*-amylase were measured by methods used previously (10). A modification of Oparin's method (3) was used to determine invertase activity.

Data were subjected to analysis of variance, where possible, and significance determined at the .05, .01, and .001 levels. In the tables "no significance" means no significance at the .05 level.

Results

Leaf regrowth was approximately two, four, and six inches by the eighth, 12th, and 16th day after top removal.

Values given in the tables are the averages of duplicate determinations made on two samples of each of the three sets of four beets dug at the various harvest dates. In the tables each set of four beets is described as a "replicate". The average of the three replicates was used to prepare the graphs. To facilitate description the letters C, F, D, and CD are used to represent control beets, beets with tops frozen and allowed to regrow, beets with tops removed by knife and allowed to regrow, and beets with tops removed by knife and all regrowth removed every second day, respectively. All percentages were based on dry weight, except where noted otherwise.

Dry Weight

The percentage dry weight, as given in Table I and Fig. 2, is based on fresh weight. The value in control beet roots declined for the first eight days, reached a minimum of 18.35%, and rose again in the second eight-day period to 20.17%, almost identical with the original value. The fall in dry weight percentage in roots of treated plants closely paralleled that of the controls for the first four days but continued to decline at the original sharp angle, reaching a minimum at the 12th day. The values rose very slightly during the final four days, becoming 16.48, 16.19, and 16.98% for F, D, and CD plants, respectively.

The analysis of variance revealed that all treatments resulted in highly significant decreases in dry weight compared with the values in the control plants. Differences in dry weight percentages caused by the variety of

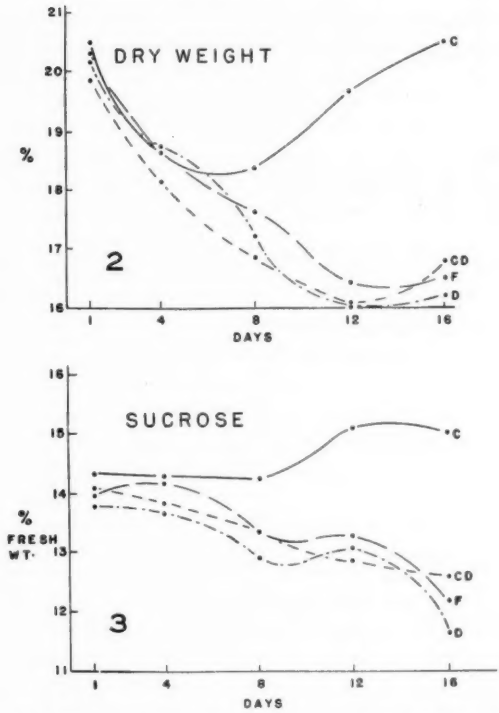
TABLE I

DRY WEIGHT AND SUCROSE OF DEFOLIATED AND CONTROL SUGAR BEET ROOTS AS PER CENT OF FRESH WEIGHT

Treatment	Replicate	Days after defoliation: Dry weight, %					Days after defoliation: Sucrose, %				
		1	4	8	12	16	1	4	8	12	16
Control	1	20.42	19.14	18.50	18.43	19.41	14.15	14.25	14.50	15.15	14.37
	2	21.04	18.40	18.43	19.55	20.85	14.30	14.10	13.65	15.35	15.00
	3	19.54	18.20	18.12	20.88	20.24	14.60	14.55	14.60	14.75	15.96
	Average	20.33	18.58	18.35	19.62	20.17	14.35	14.30	14.25	15.08	15.11
Frost defoliated	1	19.90	18.70	17.42	16.59	16.14	14.10	14.22	13.60	13.48	11.54
	2	20.70	18.60	17.58	16.00	17.20	13.75	14.18	13.20	13.15	12.57
	3	20.30	18.35	17.80	16.58	16.10	14.00	14.05	13.07	13.05	12.35
	Average	20.30	18.55	17.60	16.39	16.48	13.95	14.15	13.29	13.23	12.15
Knife defoliated	1	20.50	19.40	17.80	15.90	16.21	14.45	13.85	12.89	12.81	11.75
	2	19.70	18.00	17.00	15.39	15.74	12.80	13.65	13.53	12.98	11.20
	3	20.40	18.75	16.80	16.05	16.62	14.00	13.30	12.22	13.30	12.00
	Average	20.20	18.72	17.20	15.78	16.19	13.75	13.60	12.88	13.03	11.65
Continuously defoliated	1	20.20	18.45	17.00	15.16	16.21	14.50	14.00	12.45	12.85	11.78
	2	19.95	18.11	16.20	17.05	17.05	13.80	13.85	13.25	13.69	12.52
	3	19.80	18.04	17.32	16.06	17.68	14.00	13.55	14.13	11.95	13.48
	Average	19.98	18.20	16.84	16.09	16.98	14.10	13.80	13.28	12.83	12.59

Source of variation	D.f.	Sum of squares	Mean square	*F*	D.f.	Sum of squares	Mean square	*F*
Treatments	3	33.51	11.17	33.24**	3	23.32	7.773	28.896**
Days of harvest	4	79.81	19.95	59.37**	4	10.51	2.627	9.766**
Treatment × day of harvest	12	29.95	2.50	7.44**	12	12.24	1.020	3.792**
Error	40	13.46	0.336	—	40	10.77	0.269	—

***Significant at .01 level.*



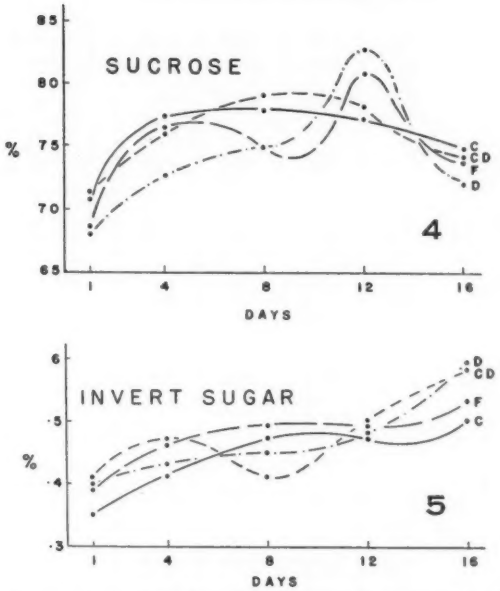
FIGS. 2 and 3. Dry weight (FIG. 2) and sucrose (FIG. 3) of defoliated and control sugar beet roots as per cent of fresh weight. C, control; F, tops frozen and allowed to regrow; D, tops removed by knife and allowed to regrow; CD, tops removed by knife and regrowth continuously removed.

treatments were not significant at the .05 level. The interaction between treatment and time of harvest was found to be significant and as shown in Fig. 2 the time of harvest had a direct bearing on the results of treatment.

Sucrose

Based on fresh weight, as is the practice in the sugar beet industry, the sucrose contents of beets given the three treatments did not differ significantly, but the percentages of sucrose were very significantly lower in these roots than in those of untreated beets. Of significance, too, was the interaction between treatments and the times of harvest. The data are given in Table I and Fig. 3.

For four days after top removal the percentage sucrose in the roots of treated plants remained on the average 0.42 lower than in the control roots, but thereafter declined with time, resulting in final values of 12.15, 11.65, and 12.59% for F, D, and CD beets respectively. The percentage sucrose in untreated beets rose 1.27 between the eighth and 12th day and on day 16 was 15.11%.



FIGS. 4 and 5. Sucrose (FIG. 4) and invert sugar (FIG. 5) of defoliated and control sugar beet roots as per cent of dry weight. Legend as for Figs. 2 and 3.

The conversion of percentage sucrose to a dry weight basis (Table II and Fig. 4) revealed that the various treatments had not resulted in a real sucrose loss. While the difference in sucrose percentage varied with time after treatment, the analysis of variance indicated that this was not the result of top removal, since none of the sucrose percentage differences was significant.

Invert Sugar

The invert sugar values, based on dry weight, are given in Table II and Fig. 5. The invert sugar increased with time in all the beets and was significantly higher in the treated plants than in the control beets. Significant differences did not result between the different treatments. The values on the 16th day were 0.50, 0.53, 0.59, and 0.58% for C, F, D, and CD beets, respectively. The rapid increase at day 10, reported by Price and Fife (7), was not detected.

Starch–Dextrin

The percentage starch in the roots of the sugar beets, as determined in the 1954 experiment, was found to be quite low, ranging from 0.21 to 0.62%, based on dry weight. Although the differences between control and treated plants were very small the content of the defoliated roots tended to be slightly lower than that of the control plants. Sixteen days after defoliation the percentages of starch–dextrin were 0.485, 0.420, 0.425, and 0.354% for C, F, D, and CD plants.

TABLE II

SUCROSE, INVERT SUGAR, TOTAL NITROGEN, AND INSOLUBLE NITROGEN OF DEFOLIATED AND CONTROL SUGAR BEET ROOTS AS PER CENT OF DRY WEIGHT

Treatment	Replicate	Days after defoliation					Days after defoliation					Days after defoliation				
		1	4	8	12	16	1	4	8	12	16	1	4	8	12	16
		Sucrose, %					Invert sugar, %					Total nitrogen, %				
Control	1	69.29	74.75	78.38	82.20	74.03	0.352	0.376	0.497	0.440	0.493	0.853	0.725	1.192	1.019	1.133
	2	67.97	76.63	74.06	78.52	71.94	0.342	0.440	0.437	0.414	0.508	1.074	0.837	0.635	0.733	0.709
	3	74.72	79.94	80.57	70.64	78.85	0.343	0.407	0.473	0.548	0.500	0.904	0.968	0.843	0.717	0.921
	Average	70.66	71.11	77.67	77.12	74.94	0.346	0.408	0.469	0.467	0.500	0.944	0.843	0.890	0.823	0.921
Frost defoliated	1	70.85	76.04	76.29	81.25	71.50	0.427	0.471	0.505	0.530	0.545	1.168	0.621	0.608	0.780	0.839
	2	66.43	76.24	75.09	82.18	73.08	0.391	0.446	0.472	0.440	0.535	0.774	0.594	0.759	0.690	0.796
	3	68.97	76.57	73.43	78.71	76.70	0.355	0.458	0.494	0.501	0.509	0.664	0.690	1.148	1.060	0.927
	Average	68.75	76.28	74.94	80.71	73.76	0.391	0.458	0.490	0.490	0.529	0.869	0.635	0.838	0.843	0.854
Knife defoliated	1	67.84	71.39	72.36	80.57	72.47	0.360	0.418	0.438	0.509	0.605	0.755	0.725	1.102	1.082	0.843
	2	64.32	75.79	79.59	84.34	71.15	0.410	0.472	0.488	0.494	0.591	0.846	1.032	0.806	0.976	0.829
	3	72.16	70.93	72.74	82.87	72.20	0.418	0.395	0.429	0.431	0.577	1.105	1.186	0.835	0.864	0.759
	Average	68.11	72.70	74.89	82.52	71.95	0.396	0.428	0.452	0.478	0.591	0.902	0.981	0.914	0.974	0.810
Continuously defoliated	1	76.52	75.88	73.24	84.76	72.67	0.430	0.477	0.394	0.561	0.530	1.055	0.721	1.135	0.884	0.659
	2	70.05	76.48	81.79	75.37	73.43	0.395	0.469	0.457	0.469	0.639	0.740	0.866	1.029	0.841	0.582
	3	66.98	75.11	81.58	74.41	76.24	0.414	0.449	0.375	0.473	0.571	0.674	1.025	0.809	0.927	0.978
	Average	71.18	75.82	78.87	78.18	74.11	0.413	0.465	0.409	0.501	0.580	0.823	0.871	0.991	0.884	0.740
												Insoluble nitrogen, %				
Control												0.091	0.084	0.079	0.085	0.090
Frost defoliated												0.073	0.058	0.063	0.064	0.060
Knife defoliated												0.093	0.071	0.084	0.067	0.078
Continuously defoliated												0.081	0.082	0.093	0.085	0.081

TABLE II—*Concluded*

SUCROSE, INVERT SUGAR, TOTAL NITROGEN AND INSOLUBLE NITROGEN OF DEFOLIATED AND CONTROL SUGAR BEET ROOTS AS PER CENT OF DRY WEIGHT—*Concluded*

Source of variation	D.f.	Sum of squares	Mean square	*F*	D.f.	Sum of squares	Mean square	*F*	D.f.	Sum of squares	Mean square	*F*
Treatments	3	23.49	7.83	0.647	3	0.0128	0.0043	3.58*	3	0.0938	0.03127	0.977†
Days of harvest	4	653.72	163.43	13.507**	4	0.1744	0.0436	36.33**	4	0.0562	0.01405	0.439
Treatment × day of harvest	12	96.88	8.07	0.667	12	0.0300	0.0025	2.08*	12	0.2464	0.02053	0.642
Error	40	483.93	12.10	—	40	0.0492	0.0012	—	40	1.2796	0.03199	—

**Significant at .05 level.*
***Significant at .01 level.*
†*No significance.*
§*No analysis of variance possible.*

Nitrogen

There were no significant differences between the total nitrogen percentages in the beets which had been subjected to the several treatments or between the total N-content of the treated and of the untreated plants. The values are given in Table II. The values for insoluble nitrogen are also included but do not permit statistical analysis. No pattern is evident in their distribution. These data are unlike those reported by Price and Fife (7), who found that in beets topped but not lifted the nitrogen value expressed as nitrogen not precipitated by copper hydroxide minus the total ammonia nitrogen showed a gradual decrease until the eighth day and then rose sharply on the 10th day.

Catalase

Catalase activity is given in Table III and Fig. 6 as the monomolecular value extrapolated to zero time and converted to a dry weight basis. The activity of this enzyme increased for the first four days and then declined steadily

TABLE III

CATALASE ACTIVITY IN DEFOLIATED AND CONTROL SUGAR BEET ROOTS AS K_0 ON A DRY WEIGHT BASIS

Treatment	Replicate	Days after defoliation 1	4	8	12	16
Control	1	0.0620	0.0782	0.0720	0.0639	0.0564
	2	0.0649	0.0800	0.0704	0.0666	0.0550
	3	0.0650	0.0780	0.0699	0.0637	0.0550
	Average	0.0639	0.0787	0.0708	0.0648	0.0554
Frost defoliated	1	0.0618	0.0782	0.0541	0.0562	0.0564
	2	0.0623	0.0791	0.0542	0.0570	0.0575
	3	0.0621	0.0766	0.0538	0.0560	0.0571
	Average	0.0621	0.0780	0.0685	0.0564	0.0570
Knife defoliated	1	0.0620	0.0784	0.0681	0.0598	0.0427
	2	0.0627	0.0778	0.0658	0.0598	0.0431
	3	0.0622	0.0756	0.0653	0.0600	0.0443
	Average	0.0623	0.0773	0.0666	0.0599	0.0433
Continuously defoliated	1	0.0642	0.0819	0.0650	0.0643	0.0600
	2	0.0640	0.0811	0.0660	0.0636	0.0593
	3	0.0638	0.0782	0.0648	0.0653	0.0578
	Average	0.0639	0.0804	0.0653	0.0644	0.0589

Source of variation	D.f.	Sum of squares	Mean square	*F*
Treatments	3	0.000378	0.00012600	120.0**
Days of harvest	4	0.003917	0.00097925	932.6**
Treatment × day of harvest	12	0.000695	0.00005792	55.2**
Error	40	0.000042	0.00000105	—

***Significant at .01 level.*

until the 16th day with the activity in the treated plants being consistently less than in the control roots. The K_0 values on the 16th day showed a greater spread than at any other time and were 0.0554, 0.0570, 0.0433, and 0.0589 for C, F, D, and CD plants.

Catalase activity in the roots of sugar beets defoliated once by frost or by knife showed a highly significant decrease when compared with control plant values. Similarly the values resulting from these two treatments differed from those in plants whose tops were continuously removed in a highly significant manner. The activity in F and D plants did not differ significantly nor did that of the CD plants and the controls. Time after treatment influenced the level of catalase activity but the highly significant effect of treatments F and D was evident.

TABLE IV

PHOSPHORYLASE ACTIVITY IN DEFOLIATED AND CONTROL SUGAR BEET ROOTS AS MICROGRAMS OF PHOSPHORUS FORMED PER MILLILITER OF EXTRACT, AND CONVERTED TO A DRY WEIGHT BASIS

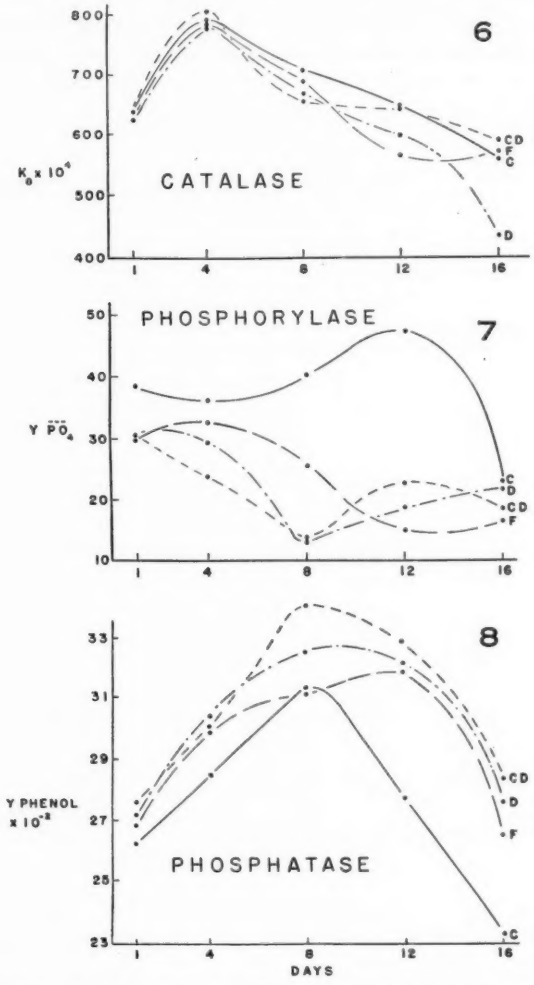
Treatment	Replicate	Days after defoliation				
		1	4	8	12	16
Control	1	33.8	37.3	38.2	45.3	25.8
	2	38.0	26.1	40.0	47.6	21.1
	3	39.4	35.5	43.0	45.5	23.2
	Average	37.1	36.3	40.4	46.1	23.4
Frost defoliated	1	31.7	34.3	24.7	15.7	13.0
	2	27.1	31.6	28.4	12.5	16.9
	3	31.7	32.5	22.5	15.7	18.6
	Average	30.1	32.8	25.2	14.6	16.2
Knife defoliated	1	29.7	27.8	12.4	15.1	15.4
	2	31.5	26.9	12.8	21.4	21.6
	3	30.9	32.3	13.2	19.3	20.0
	Average	30.7	29.0	12.8	18.6	19.0
Continuously defoliated	1	31.2	25.2	13.8	25.1	14.8
	2	30.0	22.2	13.1	24.0	18.2
	3	29.8	24.3	14.2	18.7	22.1
	Average	30.3	23.9	13.7	22.6	18.4

Source of variation	D.f.	Sum of squares	Mean square	*F*
Treatments	3	2073.11	691.04	94.92**
Days of harvest	4	1262.44	315.61	43.35**
Treatment × day of harvest	12	1566.34	130.53	17.93**
Error	40	291.06	7.28	—

***Significant at .01 level.*

Phosphorylase

The values for amylophosphorylase, expressed as micrograms of phosphorus formed per milliliter of extract, and converted to a dry weight basis, are given in Table IV and Fig. 7. Treatment resulted in a lowering of phosphorylase activity in all the plant roots. Activity on the first day after treatment was approximately 30 for each treatment and 38.5 for control plants. The values in defoliated roots fell consistently for eight days and changed but little



FIGS. 6–8. Activity of catalase (FIG. 6), phosphorylase (FIG. 7), and phosphatase (FIG. 8) in defoliated and control sugar beet roots. Legend as for Figs. 2 and 3.

thereafter. A very low phosphorylase activity in the control beets, measured on the 16th day, resulted in a very close grouping of all values at the end of the experiment.

The difference in activity of phosphorylase in control plants and that in the treated plants was significant at the .001 level. A significant difference existed between the activity in the F and the CD plants but not between values measured in the F and D sugar beets.

Phosphatase

Acid phosphatase was measured as micrograms of phenol formed per milliliter of plant extract and was converted to a dry weight basis. Table V and Fig. 8 show the values obtained. This enzyme was more active in the roots of defoliated plants than in the untreated. The greatest spread in

TABLE V

PHOSPHATASE ACTIVITY IN DEFOLIATED AND CONTROL SUGAR BEET ROOTS AS MICROGRAMS OF PHENOL FORMED PER MILLILITER OF EXTRACT, AND CONVERTED TO A DRY WEIGHT BASIS

Treatment	Replicate	Days after defoliation				
		1	4	8	12	16
Control	1	2637.7	2760.4	3096.5	2863.8	2446.2
	2	2593.2	2889.7	3131.9	2728.9	2283.0
	3	2845.9	2936.8	3157.3	2638.4	2382.4
	Average	2692.2	2862.3	3131.9	2743.7	2370.5
Frost defoliated	1	2749.2	2850.2	3136.7	3157.9	2684.6
	2	2688.4	3029.0	3122.9	3239.3	2606.4
	3	2620.6	3130.8	3088.2	3153.8	2677.6
	Average	2686.1	3003.3	3115.9	3183.7	2656.2
Knife defoliated	1	2692.7	2876.3	3164.0	3042.7	2727.9
	2	2698.0	3196.0	3271.2	3325.5	2837.3
	3	2777.7	3065.1	3321.4	3280.4	2719.6
	Average	2722.8	3045.8	3252.2	3217.4	2728.3
Continuously defoliated	1	2810.3	2892.7	3361.7	3410.3	2909.3
	2	2750.4	3016.6	3566.0	3146.6	2813.4
	3	2725.7	3062.6	3473.5	2755.3	2695.1
	Average	2762.2	2990.6	3467.1	3104.0	2805.9

Source of variation	D.f.	Sum of squares	Mean square	*F*
Treatments	3	647,440	215,813	24.19**
Days of harvest	4	2,894,700	723,675	81.12**
Treatment × day of harvest	12	428,290	35,691	4.00**
Error	40	356,821	8,921	—

***Significant at .01 level.*

activity among the treatments occurred at the time of maximum values on the eighth day. Final values were 2370.5, 2656.2, 2728.3, and 2805.9 for C, F, D, and CD beets.

The greater activity of phosphatase in the roots of treated plants than in those of the control beets was highly significant and in this instance the difference in activity resulting from treatments F and D was significant at the .05 level. Those found in the D and CD plants were not statistically different.

Beta-amylase

Beta-amylase was detected in the roots of all the plants but its activity was very low. Lack of consistency in the relation of values in the treated to those in the control plants made it impossible to arrive at any conclusion save that a similarity in the pattern of variation of the activity of this enzyme existed in the roots of the treated plants. Monomolecular reaction constants on the 16th day of the 1954 experiment were 0.75×10^{-3}, 0.30×10^{-3}, 0.33×10^{-3}, and 0.33×10^{-3} in one instance and 0.26×10^{-3}, 0.28×10^{-3}, 0.29×10^{-3}, and 0.33×10^{-3} for C, F, D, and CD respectively, in another.

Invertase

Invertase activity, expressed as milligrams of reducing sugar times 1000, is given in Table VI. The activity of this enzyme was measured during the 1954 experiment. Invertase activity in the roots of frost and knife defoliated plants showed more variation than in those of the untreated beets, rising to values two and one-half times those of the controls in the earlier part of the experiment. Values were approximately equal on the 12th day but those in the treated plants rose again by the 16th day. The activity averages for the entire period were 216, 392, and 342 for C, F, and D, respectively. The values for CD plants are not available.

TABLE VI

INVERTASE ACTIVITY IN DEFOLIATED AND CONTROL SUGAR BEET ROOTS AS MILLIGRAMS REDUCING SUGAR TIMES 10^3

Treatment	Days after defoliation					Average
	1	4	8	12	16	
Control	130	330	310	200	110	216
Frost defoliated	130	610	810	170	240	392
Knife defoliated	90	830	290	140	360	342

No analysis of variance possible.

Discussion

Peto (4) reported a fall of 0.24 in the percentage sucrose figure of sugar beets 10 days after natural freezing of the tops. The figure was the average of factory results for a 20-year period. His small-scale freezing experiment (6) showed a drop in sucrose percentage of 1.43 nineteen days after the tops were frozen in one trial and 2.3 sixteen days after freezing in a second experiment. The removal of tops by freezing and by knife resulted in similar and significant reductions in sucrose content (5). The fall was 2.88 in 12 days. These figures are similar to those reported for the present experiments wherein the drop, based on fresh weight, was 2.05 twelve days and 2.89 sixteen days after top removal.

Consideration of the sucrose percentage based on dry weight, however, does not indicate a loss of sucrose. This is evident from the lack of significance in the differences between sucrose percentage, based on dry weight, in the defoliated and control roots. The continuous decline in percentage sucrose, on a fresh weight basis, from the day of defoliation, contrasted with an increased percentage in the roots of intact plants, suggests continued absorption of water from the soil by the defoliated roots without its normal loss by transpiration. The continuous decline in percentage dry weight in all defoliated roots adds confirmation to the conclusion. Any sucrose added to the reserves by the activity of the new leaves must have been very small since it did not prevent the fall in sucrose percentage on a fresh weight basis. The absence of sucrose loss reported by Peto (4) when temperatures remained at or close to freezing for 12 days after the first killing frost may well have been the result of low temperatures on water absorption as well as on regrowth of leaves. His suggestion that the "loss" in sucrose was occasioned by the regrowth of tops is not substantiated since in this experiment no significant difference existed between values found in roots whose tops were removed and allowed to grow again and in roots whose tops were continuously removed.

The gradual increase in invert sugar with time was similar to that reported by Price and Fife (7), but it was detected in all the beet roots. The content of the treated roots was significantly greater than that of the controls. The larger invert sugar percentage in treated beets coupled with the greater phosphatase activity in the defoliated roots suggests the derivation of at least part of the invert sugar from the hexose-6-phosphates of the glycolytic process. The remainder may be the result of the greater invertase activity detected in the treated plants. The absolute amounts of invert sugar, however, were quite small and their production did not alter the sucrose content significantly.

The importance of the significantly lower catalase activity in treated roots is hard to evaluate since the part played by this enzyme, other than to prevent the accumulation of hydrogen peroxide in the tissues, is not at all clear. The lower activity may reflect a decreased rate in those respiratory reactions which produce hydrogen peroxide.

Frost, per se, did not initiate or modify enzymatic sequences in a manner unlike that effected by knife defoliation. Only in the case of phosphatase and

catalase activity did continuous defoliation result in changes which differed at the .01 or .001 level from those caused by a single defoliation with subsequent regrowth of tops. A statistical comparison of the differences in effect of the various treatments is given in Table VII.

TABLE VII

COMPARISON OF TREATMENT EFFECT ON CHEMICAL COMPOSITION AND BIOCHEMICAL ACTIVITY IN DEFOLIATED AND CONTROL SUGAR BEET ROOTS

	Treatments compared					
Difference in effect on	F† vs. C	D vs. C	CD vs. C	D vs. F	CD vs. F	CD vs. D
Dry weight	***	***	***	—	—	—
Sucrose %—fresh weight basis	**	**	**	—	—	—
Sucrose %—dry weight basis	—	—	—	—	—	—
Invert sugar	*	*	*	—	—	—
Total nitrogen	—	—	—	—	—	—
Catalase activity	***	***	—	—	***	***
Phosphorylase activity	***	***	***	—	*	—
Phosphatase activity	***	***	***	*	**	—

**Significant at .05 level.*
***Significant at .01 level.*
****Significant at .001 level.*
—No significant difference at .05 level.
†F: frost defoliated, tops allowed to regrow. D: knife defoliated, tops allowed to regrow. CD: knife defoliated, new growth removed every other day. C: untreated.

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VIRUS IN WILD PLANTS[1]

By W. D. MacClement[2] and Marilyn G. Richards[3]

Abstract

An investigation of wild plants indicates that there are very few species which are free from possible virus infection. In comparison with this, most flowering plants growing under the surface of open water show a low but significant amount of virus. The indicated level of virus infection varies throughout each summer and from one summer to the next, but is often surprisingly high. The total annual infection under the conditions employed was approximately 10% of sample population. The amount of infection in all plants varies with species and location from 0 to 50%. Total virus infection appears to be related to the growth rates of plants. The types of virus included several common to commercial crop plants, many unidentified mixtures, and some distinctly new viruses.

The literature on plant virus diseases contains many references to conditions which affect the multiplication of virus in certain test plants under controlled conditions. These diseases have been studied from the biochemist's point of view, from that of the grower, and from that of the pathologist. The emphasis in these investigations has been on the virus in question or on the crop concerned. Very little information has hitherto been gathered about the general distribution and frequency pattern of native viruses in weeds and wild plants. The total field of references for this study is extremely limited.

Brief surveys of wild plants in fields adjacent to experimental plots in Germany in 1932–33, and in England in 1948–49, indicated an unexpected high level of infection in weeds. A preliminary survey of wild plants in the Royal Botanical Gardens at Hamilton, Ontario, in 1950 and 1951, located many species showing virus-like symptoms.

From the conviction that the true relationship of virus and plant is best studied in a normal population of wild plants, and from the evidence that viruses are widely distributed through this wild plant population, it was decided to depart from the usual pattern of virus and crop plant study. A long term survey was begun to examine the frequency and distribution of virus diseases in their natural environment as found in the Royal Botanical Gardens.

Materials and Methods

The terrain of the Gardens includes many plant species found in a wide range of conditions in an area of 1800 acres. This was divided into six physiographic surface types. Three widely distributed 3-meter quadrats were established in each surface type to include most of the typical species of the area. Five specific species were selected as being representative of each physiographic area and these were used in repeated samplings. These samples

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Contribution from the Department of Biology, McMaster University, Hamilton, Ont.
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[3]*This paper is based on a thesis submitted by the junior author to the Faculty of Arts and Science in partial fulfillment of the requirements for the degree Master of Science.*

were collected from the 18 established quadrats at two-week intervals during the growing season. Samples were chosen at random without reference to appearance. Collections were made by slipping a waxed paper bag over a leaf or stem, pinching off the rest of the plant, then folding the sample into the bag without touching the leaf. The samples were macerated and inoculated immediately to test plants with a well-proved range of reaction to viruses. These were: *Nicotiana tabacum*, *Lycopersicum esculentum*, *Nicotiana glutinosa;* and, whenever space permitted their propagation, one of *Phaseolus vulgaris*, *Physalis angulata*, *Gomphrena globosa*, *Datura stramonium*, *Chenopodium album*, *Petunia* hybrids, *Spinacea oleracea*, *Cucumis cucumis*, and members of the *Brassica* family such as cabbage, turnip, mustard, cauliflower, and broccoli.

Symptoms on the test plants that were used as indicating virus infection included the following: necrotic lesions, rings, etch patterns, vein banding, mosaic mottling, chlorosis, distortion, and stunting.

The six surface types and the species sampled were as follows:

(1). *Ravine Bottom.* An accumulation of muck and silt at the bottom of a steep-sided ravine, typified by such plants as *Symplocarpus*, *Viola*, *Impatiens*, *Rubus*, and *Corylus*.

(2). *Ravine Slope.* A shaded slope above the ravine bottom type area, typified by *Trillium*, *Podophyllum*, *Arisaema*, *Rubus*, and *Smilacina*.

(3). *Uncultivated Pasture.* A pasture which is not cut or grazed, typified by *Potentilla*, *Solidago*, Gramineae, *Phleum*, and *Rubus*.

(4). *Cultivated Pasture.* Pasture which is cut once or twice a year, typified by *Trifolium*, *Arctium*, Gramineae, *Phleum*, and *Potentilla*.

(5). *Open Marsh.* Floating root mass islands surrounded by water in the marsh, typified by *Polypodiaceae*, *Salix*, *Sparganium*, *Panicum*, and *Impatiens*.

(6). *Open Water.* Two to six feet deep in Cootes Paradise; sampled plants were *Lemna minor*, *Potamogeton pectinatus*, *Potamogeton crispus*, *Ceratophyllum*, and *Nymphaea*.

Results of Virus Survey

The total virus infection varies only slightly from year to year averaging approximately 10% (Table I).

TABLE I

ANNUAL LEVEL OF INFECTION OF PLANTS IN RANDOM SURVEY AND % OF SPECIES FOUND INFECTED PER YEAR

Year	Annual level of infection	Species infected per year
1951	29 infected in 223 samples (13%)	14 out of 31 species (45%)
1952	48 " 467 " (10%)	19 " " 24 " (79%)
1953	52 " 681 " (8%)	17 " " 22 " (77%)
1954	86 " 822 " (10%)	21 " " 21 " (100%)

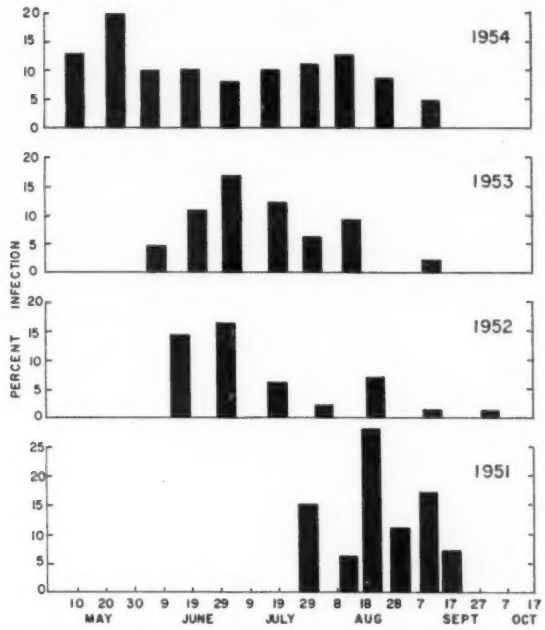

FIG. 1. Per cent of samples showing infection throughout each season.

Virus infection varies considerably from one month to the next in any one season (Fig. 1).

Virus infection on any one date varies considerably from year to year (Fig. 1). Most species of wild plants show virus infection at some time during this survey; but not all species show virus infection every year (Table I).

Species consistently showing a high percentage of infection are perennials rather than annuals (Table II).

The level of infection indicates an early maximum followed by a late maximum shifting slightly each year with weather and growing conditions (Fig. 1). The early maximum is largely made up of spring plants (Fig. 2). Such plants have a maximum infection period late in June and early in July. These are such plants as *Smilacina*, *Podophyllum*, *Trillium*, *Impatiens*, Gramineae, and *Trifolium*. Woody perennials such as *Corylus* and *Rubus* show a consistent level of infection throughout the summer.

Water plants which are found in abundance early in the summer, such as *Nymphaea* and *Potamogeton pectinatus*, show an early infection.

Those which reach a peak of growth later, such as *Ceratophyllum* and *Lemna*, show a peak infection later in the season.

Cultivated pasture has a consistently high level of infection in contrast to open marsh (Table IV).

TABLE II

FREQUENCY OF DISEASE FOUND IN SPECIES SAMPLED IN RANDOM SURVEY

Wild host	No. of times sampled	No. of times diseased	% infection
Arctium	55	7	12.7
Arisaema	52	10	18.6
Ceratophyllum	45	1	2.2
Chelidonium	5	2	40.0
Chrysanthemum	30	1	3.3
Corylus	69	10	14.5
Geranium	8	2	25.0
Gramineae	149	15	10.1
Impatiens	221	19	8.5
Lemna minor	70	6	8.6
Lysimachia nummularia	4	2	50.0
Nymphaea	64	5	7.8
Plantago mj.	3	2	66.6
Phleum pratense	123	16	13.0
Podophyllum	72	11	15.3
Polypodiaceae	62	1	1.6
Potamogeton crispus	51	2	3.8
Potamogeton pectinatus	72	3	4.2
Potentilla	141	18	12.8
Rubus	215	25	11.6
Salix	63	3	4.7
Scirpus	17	1	5.9
Smilacina	69	10	14.5
Solanaceae	4	2	50.0
Solidago	95	19	20.0
Symplocarpus	70	1	1.4
Trifolium	76	7	10.0
Trillium	71	5	7.0
Viola	71	8	11.3

The symptom picture on the test plants indicates the presence in these wild plants of many well-known virus diseases of commercial crops such as sugar beet curly top, cucumber mosaic, potato and tobacco ringspots, spotted wilt. These are seldom found as a single virus but more often as a mixture of viruses. Some symptom patterns suggest the presence of hitherto unidentified virus. This is illustrated from a sample taken from the record: *Solidago* 1953 (Table III).

Discussion

It is obvious from a study of the tables and figures presented that the amount of virus infection in wild plants varies throughout each season, and from one area to another, and in any one area from one year to the next. These variables average out in the yearly total as seen in Table I.

Within each growing season there appear to be two high points of infection (Fig. 1). Each group of plants, spring flowering, early summer flowering, late summer flowering, tends to provide its own peak of infection (Fig. 2). These peaks can be influenced by the weather conditions. Evidence can be found (Table IV) that on the ravine slope the succulent plants in well-drained

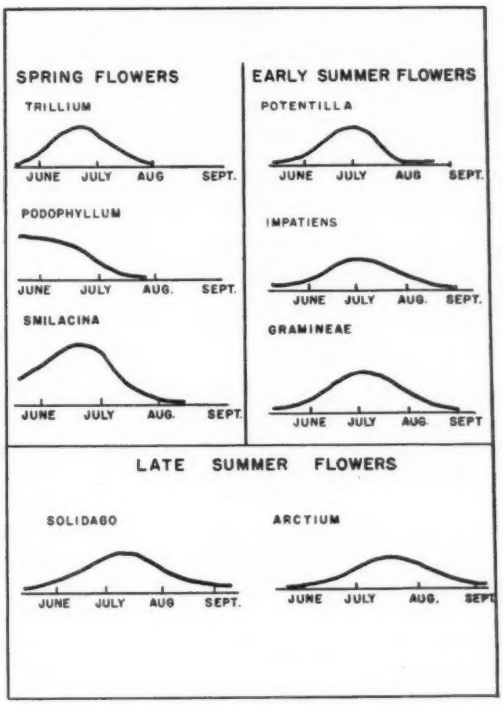

FIG. 2. Seasonal infection curves.

soil reached an early maturity in a hot humid spring of 1953 and practically disappeared in a hot dry midsummer leaving almost no source of virus for the rest of the season. This same season favored a continuous growth throughout the summer of the uncultivated late flowering pasture plants resulting in a high value of virus. This same pair of sampling areas in 1952 show a contrasting influence of a cool spring when the ravine slope plants were delayed in maturing, permitting a large accumulation of virus. The uncultivated pasture plants were only slightly influenced since their main growing season later in the summer was normal.

While the number of species showing infection in any one season varied from 45% to 100%, every species in the range of flowering plants sampled indicated the presence of virus at some time (Tables I and II).

The results suggest that two things are being measured. One is the rate of spread of infection and the other is the concentration of infection material within a particular species, and probably within an individual plant. It is known that a tobacco plant systemically infected with tobacco mosaic may in time be non-infectious. Such a phenomenon suggests that the virus has

TABLE III

VIRUS IN *Solidago* SAMPLED IN 1953

Date of sampling	Host	Symptom
June 21	*N. glutinosa*	Distortion, mottling
	L. esculentum	Distortion, mottling
	N. tabacum	Healthy
July 6 Sample 1	*N. glutinosa*	Stunting, vein banding
	P. angulata	Etch
	G. globosa	Healthy
Sample 2	*N. glutinosa*	Etch
	P. angulata	Stunting, distortion
	G. globosa	Healthy
July 20	*N. glutinosa*	Necrosis, distortion
	L. esculentum	Healthy
	G. globosa	Healthy
August 3	*N. glutinosa*	Chlorosis, mottle
	N. tabacum	Healthy
	P. angulata	Healthy
August 17	*N. glutinosa*	Distortion
	L. esculentum	Distortion
	P. angulata	Healthy

become so dilute as to be non-available (1) or that, because of host reactions, has become altered and is latent, such as the vegetative stage of bacteria phage (2). This occurrence is found only when the plant has ceased vegetative growth. The implication would seem to be that what has been measured is the state of virus within the flora, as well as its distribution and frequency. This is a dynamic state, in which the measured level of infection increases at a rate related to the rate of growth and total growth of the plant, then decreases with maturation and cessation of vegetative growth. The examples of individual infection curves expressed in Fig. 2 would seem to agree with this.

The surveys reported here account only for viruses which are mechanically inoculable and which show a reaction on the limited number of test plants used. Even on this basis the indication is that the average level of infection in any summer is approximately 10% of the plant population. This figure no doubt could be doubled if one also takes into account the multitude of viruses transmitted only by insect vectors.

The chance of a susceptible plant becoming infected with virus is subject to a series of variables. Since insects are assumed to be the most important means of transmission, any variable affecting them will be of primary interest. The over-all population of transmitting insects not only varies from year to year, but from month to month within any one area. This population also varies considerably from one area to another at any one time. In addition, the movement of small insects within one area will be influenced by wind strength and direction and density of plant population.

TABLE IV

INCIDENCE OF VIRUS PER PHYSIOGRAPHIC AREA

Type of area	% plants sampled showing reaction			
	1952	1953	1954	Av. for area
Ravine bottom	11.8	9.7	6.9	9.5
Uncultivated pasture	9.2	15.8	11.2	12.1
Cultivated pasture	16.1	9.8	11.7	12.5
Ravine slope	14.7	3.9	13.5	10.7
Open marsh	4.2	1.8	8.5	4.8
Open water	5.2	4.5	11.0	6.9
Average for year	10.2	7.6	10.4	9.4

Since the rate of spread of virus appears to be indirectly, but nevertheless significantly, influenced by many environmental factors such as temperature, humidity, rainfall, and directly by density of population of suitable hosts and of insect population, slight variation or coincidence of variable will affect the amount of virus found in samples.

It is now established that wild plants are susceptible to a wide range of viruses. Often one species shows reactions indicating susceptibility to more than one virus. The next logical step, i.e., positive identification of these viruses, is now underway.

Acknowledgments

The authors are indebted to the Research Council of Ontario and to the Ontario Research Foundation for the research grants which supported this work.

Grateful appreciation is also expressed to the director and assistants of the Royal Botanical Gardens for their aid in this program.

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MATERNAL INHERITANCE OF LEAF VARIEGATION IN HEXAPLOID WHEAT[1]

By T. J. Arnason[2]

Abstract

A P^{32}-treated *vulgare* wheat plant produced an offspring having yellow-green stripes on the leaves. The variegated leaf character is maternally inherited. Progenies of individual variegated selfed plants differ widely in the proportions of green : variegated individuals. Green sibs of variegated plants breed true. In crosses between variegated awn-tipped and green bearded plants, the awn character segregated in a simple Mendelian fashion while the variegation appeared only in individuals whose maternal parent was variegated. Plastid mutation independent of chromosomal genes is presumed to have occurred in the P^{32}-treated parent of the original variegated plant. On the plastid-mutation hypothesis, variegated plants have two kinds of plastids, normal and mutant, and either or both kinds may be represented in the eggs produced. When both kinds of plastids are present, segregation during embryo development may result in the variegated effect seen in the older plants.

Introduction

Most of the hereditary leaf color characters of angiosperms are produced by the action of nuclear genes. Many simply-inherited chlorophyll variations have been described, for example in corn (8) and barley (9). However, since plastids are capable of perpetuating their kinds by division, it is not surprising to find some hereditary plastid modifications that are not under the control of specific chromosomal genes. When this happens, the mutated plastids are transmitted only via the mother to offspring. At least in some cereals the sperm brings in little or no cytoplasm at fertilization (3). It is supposed (3, 4, 7) that variegated plants having both normal and mutated plastids may produce eggs having plastid primordia of either or both kinds. When both kinds of plastids are present in a zygote, segregation during development of the embryo may be responsible for the variegated appearance of the leaves in the seedling or older plant.

Maternally inherited variegation in cereals has been reported to occur in wheat (6), barley (1, 4), and corn (7). The plastid mutations of iojap corn and of variegated barley are induced by nuclear genes.

A stripe-leaved plant was observed in 1949 among the offspring of a P^{32}-treated wheat plant (*Triticum aestivum* L. em. Thell. subspp. *vulgare* (5)) of the Thatcher variety. The roots of the parent plant had been supplied with 65 mrd. of P^{32} 40 days after seeding and the mutation was, therefore, attributed to the action of absorbed radioactive phosphorus. The self-pollinated variegated plant produced some green and some variegated offspring. An investigation of the method of inheritance was undertaken with results as reported in this paper.

[1]*Manuscript received June 11, 1956.*
Contribution from the Department of Biology, University of Saskatchewan, Saskatoon, Sask.
[2]*Professor of Biology.*

The Variegated Wheat Plants

Variegated leaves have a striped appearance with dark green and yellow or light yellow-green bands alternating. Stripe widths vary a good deal as may be seen in the leaves represented in Fig. 1. Variegated plants may have both striped and normal green leaves. Generally the striping is more prominent in plants approaching maturity than in seedlings. Variegated leaves are softer, limper than normal green leaves.

The variegated plants mature less rapidly than their green sibs. Plants having large mutant and small normal segments often do not form fruit and may even fail to grow beyond the three- or four-leaf stage. The yellow-green portions are therefore presumed to be relatively inefficient in photosynthesis.

In paraffin sections of variegated leaves it was observed that the leaves were thinner and the number of mesophyll-cell layers reduced in the yellow-green segments. In appearance the plastids of these mesophyll cells were normal except for an apparent slight reduction in size (average diameter yellow-green 4.5 μ; dark green 6 μ).

Meiotic divisions were studied in several hundred microspore mother cells of variegated plants and of F_1 (variegated $\times$ green) hybrids. With rare exceptions, countable metaphase-1 cells had 21 bivalent chromosomes. Meiotic anaphases also were regular in appearance. It is unlikely, therefore, that the variegation is related to a large duplication, deficiency, or other gross chromosomal change.

The Inheritance of Variegation

The original variegated plant, self-pollinated, produced four variegated and nine green offspring. The green plants of this and subsequent generations bred true, but the variegated plants did not. In each generation, nearly all of the variegated plants have produced offspring of two kinds, green and variegated. Each of the individual plant progenies grown has usually been restricted to a single 4-ft. row (16 plants). In these small progenies the proportion of green : variegated has ranged from 16 : 0 to 0 : 16.

The irregular transmission of the variegation from parents to offspring is illustrated in Table I. The 38 representative progenies listed in the table include 497 plants of which 236 or 47.5% are variegated. The 1 : 1 ratio approached in the total must be considered to be of no significance. Certainly few of the individual progenies approximate this ratio. Although occasionally all of the offspring of a particular parent are variegated, it has not been possible to isolate a true-breeding variegated line.

Reciprocal crosses between variegated and normal Thatcher wheat were made. The phenotypes of more than 50 F_1 plants and their progenies were recorded. When the female parent was green, all the F_1 and F_2 plants were green. From the cross variegated ♀ $\times$ green ♂ the F_1 generation consisted of 28 green and 48 variegated plants. From 26 green F_1 plants, 644 green and 0 variegated plants were obtained. The F_2 generation from 25 variegated F_1 plants consisted of 105 green and 67 variegated plants.

PLATE I

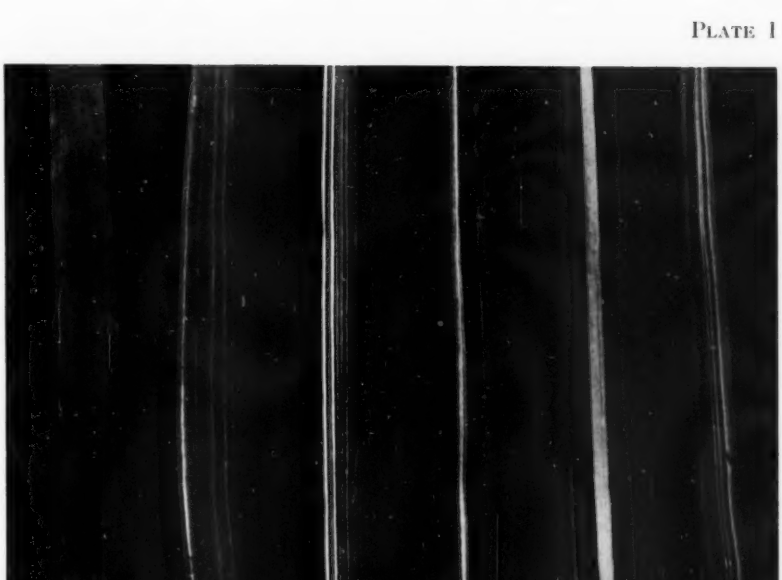

FIG. 1. Variegated leaves of *vulgare* wheat. Green leaf at left, others variegated. Note the variability in the widths and positions of yellow-green bands.

To prove that variegation is maternally inherited, crosses with a variety having distinctive phenotypic markers of its own were made. A bearded mutant of the Thatcher variety, obtained after P^{32} treatments (2), was used in crosses with variegated plants. The F_1 and F_2 results are given in Table II. As may be seen from the table, there is simple Mendelian inheritance of the beard vs. tip-awn character pair but non-Mendelian maternal inheritance of variegation.

TABLE I

SEGREGATION IN PROGENIES OF INDIVIDUAL SELF-POLLINATED VARIEGATED WHEAT PLANTS

1953 pedigree number	Green	Varieg.	% varieg.	1954 pedigree number	Green	Varieg.	% varieg.
498	3	7	70.0	487	11	4	26.7
499	14	0	0.0	488	3	11	78.6
500	0	11	100.0	489	3	10	76.9
501	2	10	83.3	490	8	6	42.9
502	11	4	26.7	491	0	10	100.0
503	15	0	0.0	492	4	10	71.4
504	15	0	0.0	493	6	7	53.8
506	6	8	57.1	495	1	11	91.7
507	10	5	33.3	496	9	4	30.8
508	15	0	0.0	500	6	8	57.1
509	9	5	35.7	501	4	7	63.6
510	6	9	60.0	503	4	6	60.0
512	14	1	6.7	504	3	9	75.0
513	13	0	0.0	505	1	14	93.3
518	8	5	38.5	506	2	11	84.6
519	15	0	0.0	507	1	11	91.7
520	6	5	45.5	508	5	7	58.3
521	6	6	50.0	509	5	5	50.0
524	2	9	81.8	980	15	0	0.0
Totals	170	85	33.3		91	151	62.4

TABLE II

CROSSES BETWEEN GREEN BEARDED AND VARIEGATED TIP-AWNED PLANTS

Parents	F_1		F_2			
	Green tip-awned	Varieg. tip-awned	Green tip-awned	Green bearded	Varieg. tip-awned	Varieg. bearded
Green bearded ♀ × Varieg. tip-awned ♂	28	0	179	77	0	0
Varieg. tip-awned ♀ × Green bearded ♂ *	7	11	45	12	46	14

**The F_2 of this cross is from variegated F_1 plants.*

Discussion and Conclusions

If a chromosomal determiner of variegation is postulated, one must assume that this also causes inviability of microspores or male gametophytes. Variegated plants would then be heterozygous and no homozygous yellow-green individuals would be expected. However, the great irregularity in the transmission of variegation makes it appear unlikely that a chromosomal mechanism is involved. While a 1 : 1 ratio is approached in some totals, it is not at all evident when individual progenies are listed separately. The cytological observations also have failed to provide evidence of any aberrant chromosome condition.

The unit governing variegation in these wheat plants is most likely extranuclear since it is transmitted by eggs but not by sperms. Defective plastids or proplastids are probably present in eggs destined to develop into variegated plants. Since variegated plants have both normal and mutated plastids, it is possible that plastid segregation results in some eggs having only normal, some having mixed, and some having only mutant plastids. Variable proportions of green and variegated plants occur as expected on this hypothesis but self-colored yellow-green plants have not been observed. Perhaps reverse mutation happens with a frequency sufficiently high to ensure that all viable zygotes of variegated plants have some normal plastids.

Acknowledgments

Financial support provided by the National Research Council of Canada is gratefully acknowledged.

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DECAY IN SUBALPINE SPRUCE ON THE ROCKY MOUNTAIN FOREST RESERVE IN ALBERTA[1]

By D. E. Etheridge[2]

Abstract

Data from 456 living subalpine spruce on six 0.1-ac. plots in the Bow River Forest in 1950 and six 0.2-ac. plots in the Crowsnest, Bow River, and Clearwater Forests in 1952 show a total of 203 separate infections of which approximately half occurred in the basal part of the trees. Most of the butt rot was associated with *Polyporus circinatus* var. *dualis* Peck, *Flammula connissans* Fr., and an unidentified fungus designated "Unknown C". *Coniophora puteana* (Schum. ex Fr.) Karst. was the fungus associated with the major portion of the brown butt rot. Among the white trunk rots, *Stereum sanguinolentum* Alb. & Schw. ex Fr. was the fungus most frequently isolated from infected trees and *Fomes pini* (Thore) Lloyd was responsible for the largest cull losses. *Peniophora septentrionalis* Laurila, which was isolated from *Picea glauca* (Moench) Voss and *P. engelmannii* Parry, was the third most important fungus associated with trunk rot. Trunk rots account for 70% of the decay losses while fungi producing white rots account for 93.6% of the total decay. The incidence of decay increased progressively with age at different rates for trees on "dry" and "moist" sites. The two sites are characterized by distinctive ground cover associations.

Introduction

The first investigation of decay in subalpine[3] spruce was undertaken at the Kananaskis Forest Experiment Station, Seebe, Alberta, in 1950 by members of the Forest Pathology Laboratory, Saskatoon (4, 10). The object was to provide precise decay data to the Forestry Branch, Department of Northern Affairs and National Resources, Ottawa, for their experiment in partial cutting of white spruce (9), but it served also as the beginning of a more comprehensive investigation to determine the decays and decay losses in subalpine spruce on the Rocky Mountain Forest Reserve. With the establishment of a Forest Pathology Unit at Calgary in 1952, the continuation of the project was assigned to this Laboratory and additional sampling in spruce was carried out during the summer of 1952. This report deals with investigations of the fungi responsible for decays of subalpine spruce and presents data on the occurrence and relative importance of each fungus.

The 1950 and 1952 investigations were conducted in the Clearwater, Bow River, and Crowsnest Forests in the Eastern Rockies Forest Conservation area (Fig. 1). These three Forests comprise the Rocky Mountain Forest Reserve and cover an area of 5335 square miles[4] of which 87% is productive forest land. The region carries an estimated 4,700,000,000 cu. ft. of softwood timber, lodgepole pine being the dominant species in the Clearwater and Bow River Forests; in the Crowsnest Forest the volumes of spruce and Douglas fir about equal the pine volume (3). Utilized forests of the region are predominantly spruce and usually occupy the lower levels of the main valleys

[1]*Manuscript received June 11, 1956.*
Contribution No. 309 from the Forest Biology Division, Science Service, Department of Agriculture, Ottawa, Canada.
[2]*Forest Pathology Laboratory, Calgary, Alberta.*
[3]*Subalpine spruce refers collectively to the following species of spruce found in the Rocky Mountain Forest Reserve: Picea glauca (Moench) Voss, P. mariana (Mill.) B.S.P., P. engelmannii Parry, and P. glauca (Moench) Voss var. albertiana (S. Brown) Sarg.*
[4]*Below 6000 ft. above sea level (includes the Kananaskis Forest Experiment Station area).*

of the east slope watershed. The elevation of the region varies between 4400 and 8000 ft. above sea level with the timber line at approximately 6500 ft. The climate is characterized by a short growing season, relatively high precipitation largely in the form of snow, and wide diurnal and seasonal range of temperature.

Method of Study

During the two field seasons a total of 456 living subalpine spruce trees were felled and critically examined on 12 sample plots in the three Forests of the Reserve. The localities at which sample plots were established are shown in Fig. 1. Except for some minor modifications which were noted, the field methods followed in 1952 were basically similar to those followed in 1950. The six plots established at Kananaskis in 1950 were 0.1 ac. in size and those established in 1952 each contained 0.2 ac. All the trees of 2.6 in. d.b.h.[5] and over on the 0.1 ac. plots were felled and sectioned, while only the spruce trees having a d.b.h. greater than 4 in. were felled on the 0.2 ac. plots.

Trees were felled at the height of 1 ft. and cut into 4 ft. sections. In 1950 the sections were more often cut into standard log lengths except where it was necessary to dissect them further to trace the extent of decay. The length and diameter of each log section and the dimensions of the rot were measured and recorded to permit the computation of gross and decay volumes in the laboratory. Individual tree volumes representing the gross and decay volumes were computed either by Smalian's formula[6] or by plotting the tree and decay dimensions on tree measurement form SB 239. The latter computations involved measuring the area between the outline of the plotted tree on the form with a planimeter and multiplying the results by a conversion factor to obtain the volume in cubic feet. Decay volumes represent the total volume of decayed wood present in the stem above the ground. Cultures were made from the decayed wood if the causal fungus was not immediately recognized from the type of decay; isolates of unknown rots were taken to the laboratory for identification by cultural methods.[7]

The age of each tree was determined from annual ring counts made on the stump or at the d.b.h. section. Where this was found impossible owing to the presence of advanced decay, counts were made at a height where a complete ring count was possible. If the merchantable stem was completely decayed, e.g. *Fomes pini* (Thore) Lloyd "cull trees", the age of the tree was assumed to be that of an adjacent sound tree of similar size and ring pattern.

[5]*Diameter at breast height outside bark.*

[6]*Smalian's formula: volume* $= \frac{(B + b)}{2} L$; B = *area of base, sq. ft.,* b = *area of top, sq. ft.,* L = *length, ft.*

[7]*The author is indebted to Dr. M. K. Nobles, Senior Mycologist, Division of Botany and Plant Pathology, Ottawa, who identified or confirmed the identification of many of the fungi encountered in this investigation.*

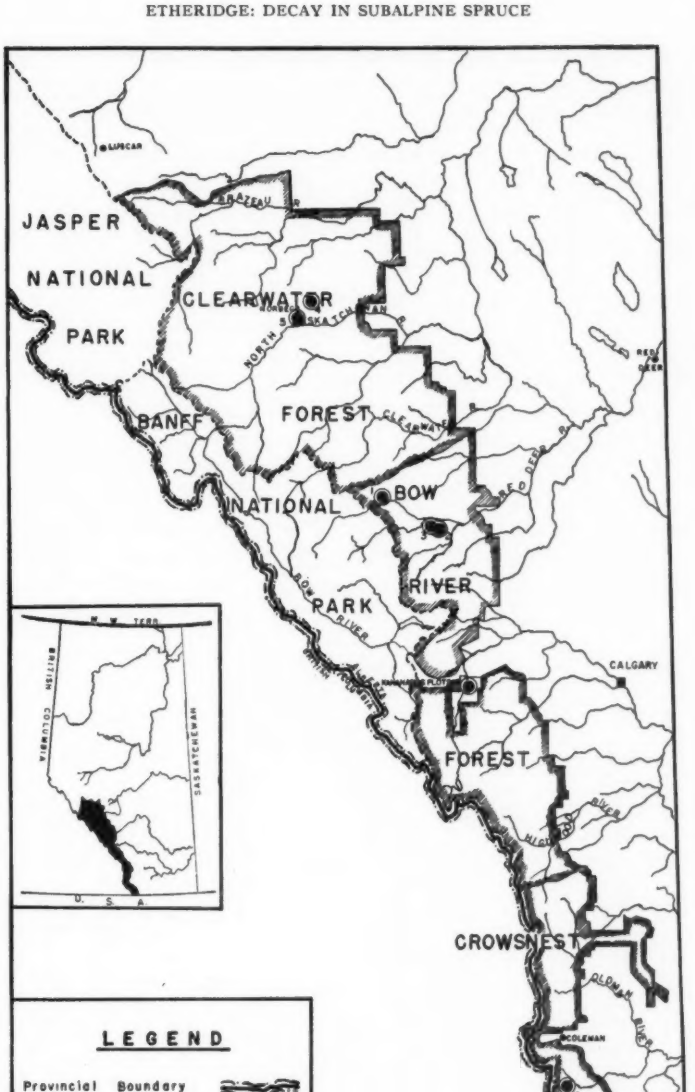

FIG. 1. Map of Alberta showing the area covered by the Rocky Mountain Forest Reserve and the location of the sample areas established in 1950 and 1952.

From the ring counts, total ages were computed for each tree by applying an appropriate age factor determined from an age–height curve based upon data obtained from dominant seedlings collected from all the sample areas.

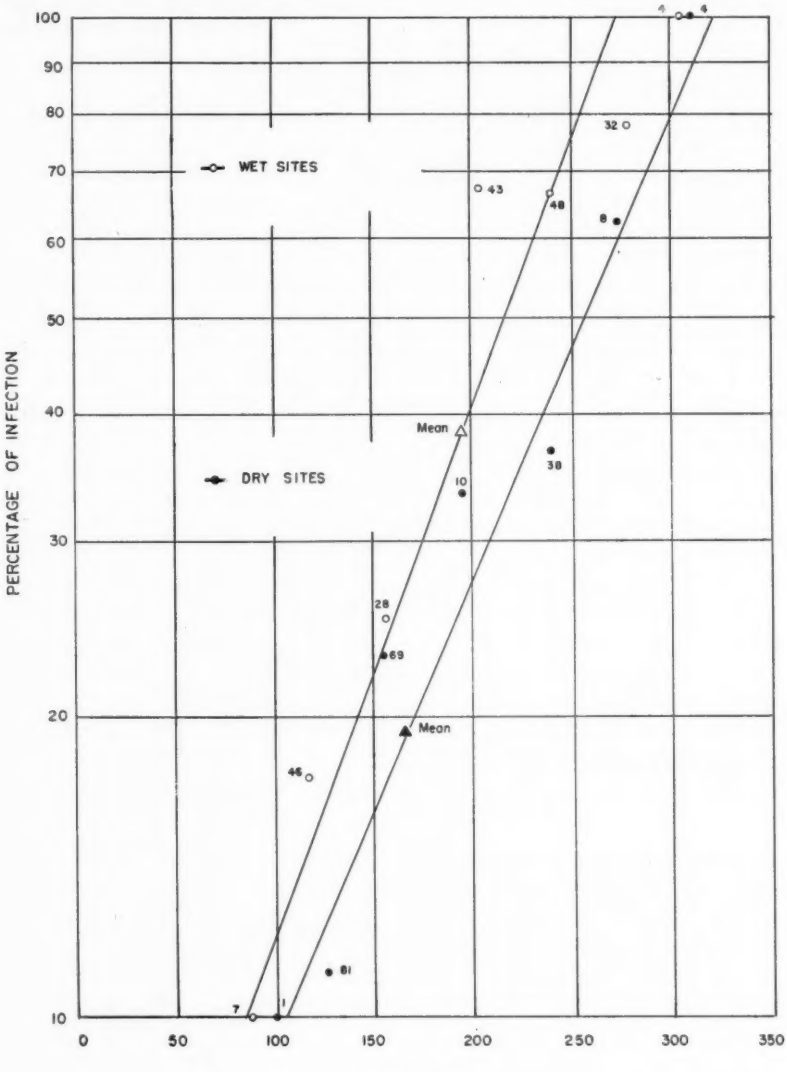

FIG. 2. The relation of tree age to infection by decay fungi in subalpine spruce on "moist" and "dry" sites in the Rocky Mountain Forest Reserve.

Relation of Infected Trees to Age and Site

The incidence of decay in living subalpine spruce is shown in Fig. 2 for trees on two different sites. The two site classes used for this analysis were determined on ecological criteria which were developed from records of the important ground cover plants that characterized the different sample plots. These records were made originally to assist in the classification of the stands for descriptive purposes, but upon further examination of the data two distinctive plant associations were recognized which appeared to be consistently associated with dry or moist sites. Accordingly, on the basis of certain plant indicators, the plots were separated into dry and moist site classes, and the incidence of infection was determined for each. The specific plant indicators found consistently associated with the dry sites in this survey were *Shepherdia canadensis* (L.) Nutt., and *Arctostaphylos uva-ursi* (L.) Spreng. (Figs. 4 to 6). The plants used to distinguish the moist sites were *Mertensia paniculata* (Ait.) Don., *Smilacina amplexicaulis* Nutt., *S. stellata* (L.) Morong., and *Actaea rubra* (Ait.) Willd. (Figs. 7 to 9).

Site quality also was found to be related to the plant associations. An analysis of the volume data showed that the average gross volume for trees on the moist sites was about twice that obtained for trees of similar ages on the dry sites.

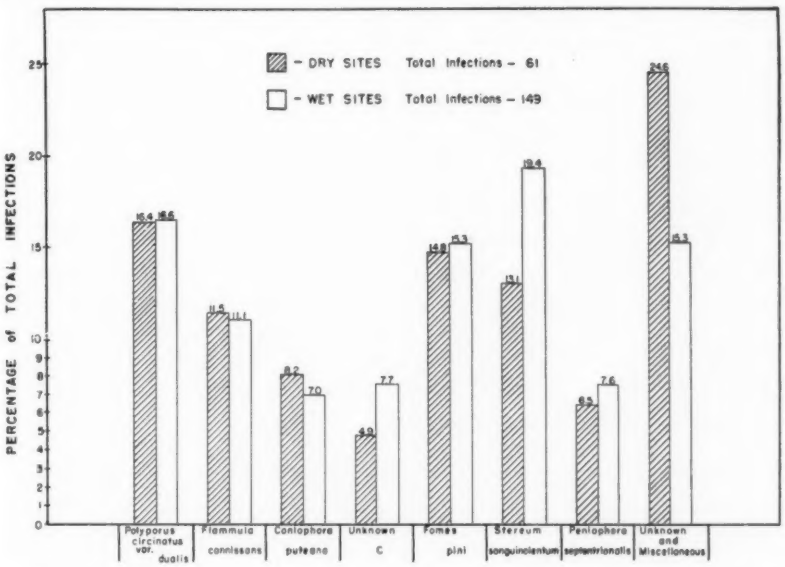

FIG. 3. Relative frequencies of occurrence of the wood-destroying fungi expressed as a percentage of the total number of infections in diseased trees on "dry" and "moist" sites.

The regressions in Fig. 2 indicate consistent trends of increasing infection with age on both sites, with a higher percentage of trees infected on the moist sites in all age classes. A comparison of the means, which are significantly different at the 1% level of probability, reveals that twice as many infected trees may occur in the stands on the moist sites as on the dry sites. All the survival trees on the moist sites over 275 years had decay while on the dry sites, the incidence of decay reached 100% at 325 years. On the basis of the total sample, the data suggest that subalpine spruce on the Reserve may attain an age of 100 years before infection[8] occurs. Because these curves are based on averages of individual survival trees for the different age classes, the data make no provision for normal mortality in the stand which would remove infected trees at different ages. Thus, on a stand basis the incidence of infected trees might be higher at an earlier age than the curve values indicate.

Since it was possible that the higher incidence of infected trees on the moist sites was due to the presence on these sites of more destructive species of fungi, a comparison was made of the fungi responsible for the infections on each site. The frequency of occurrence of the principal decay fungi on the two sites is graphically illustrated in Fig. 3.

It is evident from the sample (Fig. 3) that the frequency of occurrence of the decay fungi on subalpine spruce on the two sites is relatively uniform. Only two fungi, "Unknown C", a butt-rotting fungus, and *Stereum sanguinolentum* Alb. & Schw. ex Fr., causing a trunk rot, occur more frequently on one site than the other; both of these fungi occur more frequently on the moist sites. The remainder of the fungi, including *Fomes pini* and *Polyporus circinatus* var. *dualis* Peck, which together account for over half the volume of decay in the total sample, were equally important on the two sites. It is unlikely, therefore, that the higher incidence of infection (144 infections on the moist sites against 61 on the dry sites) or the greater decay losses found on the moist sites is the result of more destructive species of fungi predominating on these sites. On the other hand, there appears to be a connection between site moisture conditions, as determined by the associated ground vegetation, the rate of tree growth, and the incidence of infection. It appears from this relationship that the faster-growing trees might be more prone to infection than the slower-growing trees.

[8]*Infection determined on the basis of decay visible at stump height or higher.*

FIG. 4. A typical even-aged stand of white spruce on a "dry" growing site in the Rocky Mountain Forest Reserve.

FIG. 5. Characteristic plants of *Juniperus communis* L. and *Shepherdia canadensis* representative of the "dry" site ground vegetation.

FIG. 6. Characteristic plants of *Shepherdia canadensis* and *Arctostaphylos uva-ursi* representative of the "dry" site ground vegetation.

PLATE I

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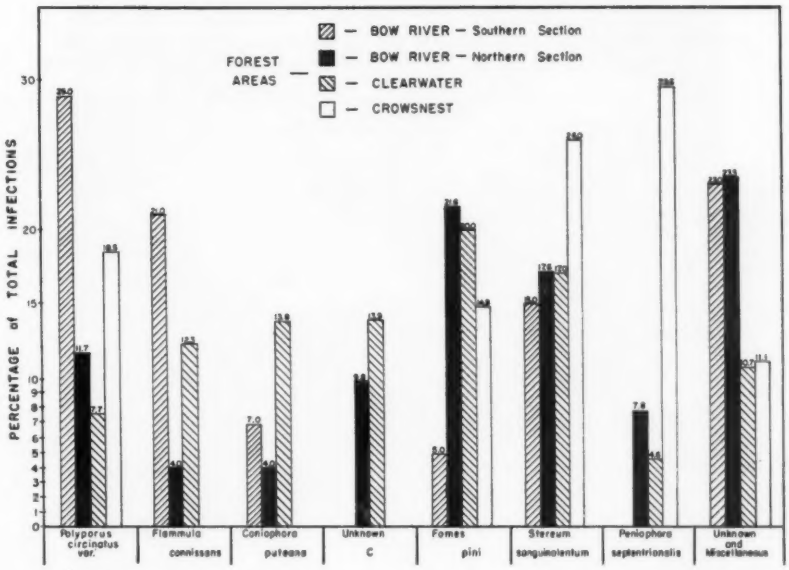


FIG. 10. Relative frequencies of occurrence of the wood-destroying fungi expressed as a percentage of the total number of infections of diseased trees in each of the three Forests.

Fungi Causing Decay in Living Subalpine Spruce

The occurrence and relative importance of the wood-destroying fungi encountered in this survey are shown in Table I. The frequency of occurrence of the fungi in the three Forests of the Reserve is recorded graphically in Fig. 10.

The decays were separated into white and brown rots, depending on the characteristic appearance of the advanced rot. It was also convenient to separate the decays into basal rots and trunk rots. In the former, no distinction has been made between fungi that normally gain entry by means of the roots and those that enter the heartwood from wounds in the basal section. All the fungi which were associated with decay that was confined to the stump section, or decays that decreased in volume immediately above this section, were classed as root- and butt-decaying fungi. Trunk-decaying

FIG. 7. A typical even-aged stand of white spruce on a "moist" growing site in the Rocky Mountain Forest Reserve.

FIG. 8. Characteristic plants of *Smilacina amplexicaulis* representative of the "moist" site ground vegetation.

FIG. 9. Characteristic plants of *Mertensia paniculata* representative of the "moist" site ground vegetation.

TABLE I

OCCURRENCE AND RELATIVE IMPORTANCE OF THE DIFFERENT WOOD-DESTROYING FUNGI IN LIVING SUBALPINE SPRUCE ON THE ROCKY MOUNTAIN FOREST RESERVE IN ALBERTA

Fungus	No. of infections	Percentage of total infections	Volume of decay (cu. ft.)	Percentage of total decay volume
White root and butt rots	78	38.40	177.61	27.05
Polyporus circinatus var. *dualis* Peck	35	17.24	108.97	16.59
Flammula connissans Fr.	22	10.83	42.46	6.46
"Unknown C"*	13	6.40	20.83	3.17
Stereum sulcatum Burt	1	0.49	2.24	0.34
Armillaria mellea (Vahl ex Fr.) Quél.	1	0.49	Trace	
Undetermined	6	2.95	3.11	0.47
Brown root and butt rots	20	9.85	21.75	3.31
Coniophora puteana (Schum. ex Fr.) Karst.	11	5.41	12.83	1.95
Polyporus balsameus Peck	1	0.49	1.30	0.19
Undetermined	8	3.94	7.62	1.16
White trunk rots	97	47.85	437.01	66.55
Fomes pini (Thore) Lloyd	31	15.27	225.79	34.38
Stereum sanguinolentum Alb. & Schw. ex Fr.	38	18.71	91.95	14.00
Peniophora septentrionalis Laurila	17	8.37	73.91	11.25
Undetermined	11	5.41	45.36	6.90
Brown trunk rots	8	3.90	20.22	3.09
Trametes serialis Fr.	3	1.47	4.12	2.15
Omphalia campanella Fr.*	1	0.49	1.39	0.21
Trametes heteromorpha (Fr.) Bres.	1	0.49	2.05	0.31
Lensites saepiaria Wulf. ex Fr.	1	0.49	0.50	0.07
Undetermined	2	0.98	2.15	0.32
Total	203	100.00	656.59	100.00

**Some doubt exists whether this fungus actually causes decay in living trees since it was associated with both white and brown rots.*

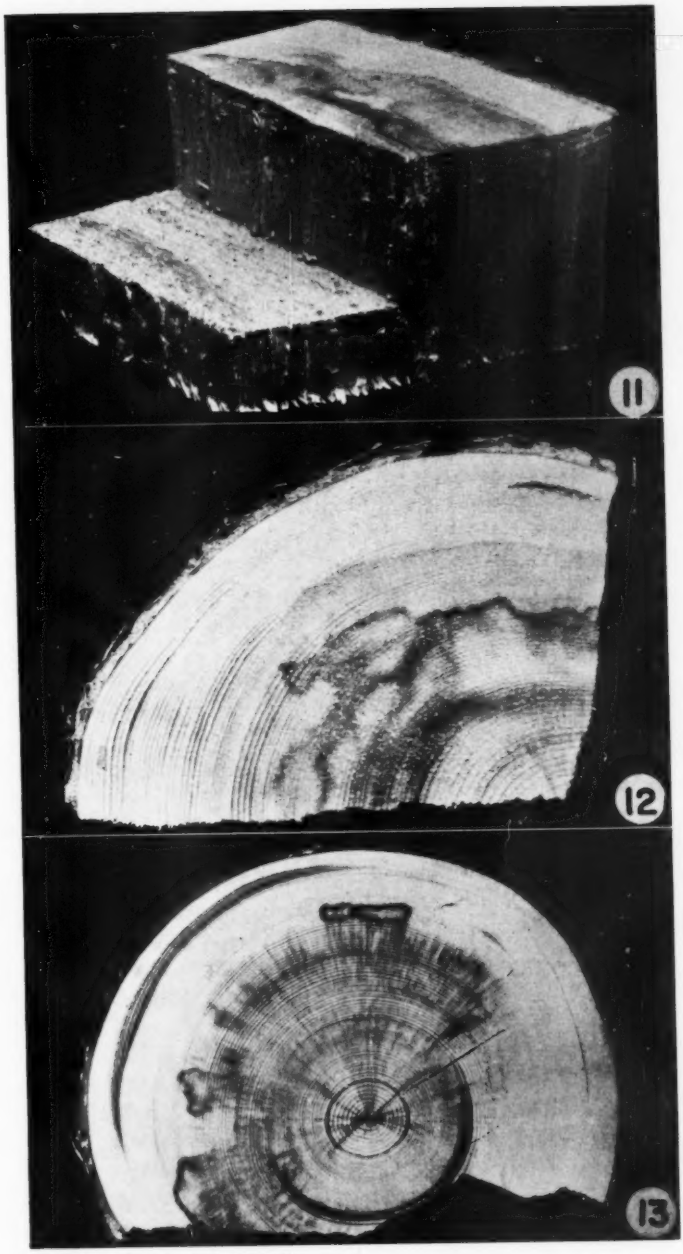
fungi, on the other hand, are defined as fungi found associated with decays occurring in the upper portion of the tree, but include some heartrot fungi that are also butt-infecting organisms, e.g. *Fomes pini*, which may under favorable conditions extend progressively downward in the heartwood to invade the roots of infected trees.

In this survey, almost half of the total number of infections occurred in the basal portion of the trees. Among the fungi associated with basal rots, *Polyporus circinatus* var. *dualis* causing a white pocket rot (Fig. 11) occurred most frequently and was responsible for 17% of the total infections. Undoubtedly, this fungus is of prime importance as the cause of butt rot in

FIG. 11. Decay caused by *Polyporus circinatus* var. *dualis* in Engelmann spruce showing the advanced white-pocket stage of the typical rot. The firm red stain of the incipient stage is surrounded by a gray border on the transverse and longitudinal sections.

FIG. 12. Decay caused by *Peniophora septentrionalis* in the heartwood of living Engelmann spruce. The firm grayish-brown stain of the incipient stage is surrounded by an irregular blue-gray border on the transverse section.

FIG. 13. Decay caused by *Peniophora septentrionalis* in the heartwood of living white spruce. The decay is frequently characterized near the sapwood by irregular brown stain pockets surrounded by a blue-gray border and throughout the heartwood by thin radiating lines of rot which appear to follow the wood rays.


11
12
13

subalpine spruce on the Reserve. Most of the remaining butt rots were associated with *Flammula connissans* Fr. (11%), *Coniophora puteana* (Schum. ex Fr.) Karst. (5%), and an unidentified fungus designated by Dr. Mildred K. Nobles as "Unknown C" (6%).

The occurrence of *Flammula connissans*, the second most frequently isolated fungus of subalpine spruce, is of interest since it has been reported recently as a cause of white root rot in living spruce and lodgepole pine in Alberta (4, 10). The present investigation confirms the findings of these earlier studies regarding the importance of *F. connissans* as a major root-rotting fungus of spruce. This fungus, however, may occur sporadically in some localities as shown by its infrequent isolation from plots in the northern part of the Bow River Forest and its absence on the study area located in the Crowsnest Forest.

Coniophora puteana, important as a destroyer of building timbers, both here and in Europe, is becoming increasingly prominent as the cause of brown cubical butt rot in numerous North American coniferous hosts. This fungus was the major cause of brown butt rot in white and black spruce on the Reserve and has been recently reported from lodgepole pine in the Clearwater Forest in Alberta (8). Its presence as an important butt rot fungus in alpine fir in this region is anticipated since it has been reported from this host in the Upper Fraser region in British Columbia (2). *Coniophora puteana* is also responsible for over 40% of the brown cubical butt decays in balsam fir, a closely related species, in Eastern North America (1).

The frequent occurrence of "Unknown C", associated with a yellow stringy butt rot, is also of interest. This fungus, which remains unidentified, apparently has not been previously reported as a major decay organism. It was responsible for over 6% of the total number of infections despite its rather limited distribution among the study areas. According to the positive reaction shown by cultures of this fungus when grown on tannic acid medium, it has been classified with the white-rot fungi.

Fungi producing the white trunk rots were the organisms most frequently isolated from subalpine spruce. Most of these infections were caused by *Stereum sanguinolentum* (19% of the total number), *Fomes pini* (15%), and *Peniophora septentrionalis* Laurila (8%) (Figs. 12, 13). Although the highest number of infections were caused by *Stereum sanguinolentum*, it is interesting that over one-third of the total decay volume was referred to *Fomes pini*. Because *S. sanguinolentum* is an important slash-destroying organism, the presence of an abundant supply of inoculum in the forest might explain the large number of infections ascribed to this fungus. Both these fungi, however, have been widely reported as major decay pathogens on coniferous hosts throughout the Northern Hemisphere, and their apparent unrestricted range over the study area indicates they may be considered ubiquitous in the Reserve.

Peniophora septentrionalis, the cause of a white trunk rot, has been previously reported by Denyer and Riley (4) as occurring on spruce in Alberta, but at the time of their report, the identification of the fungus was considered to be uncertain. It has also been reported under the provisional designation "Unknown M" (5). Although the identification of *P. septentrionalis* and its occurrence on spruce in Alberta and elsewhere in Canada have now been definitely established by Nobles (7), the decay caused by this fungus has not been previously described in detail. Evidence obtained from an examination of the decay associated with this fungus on subalpine spruce indicates that both the incipient and late stage of the rot appear to be manifested by an irregular grayish brown stain in the heartwood; the wood remains firm (Figs. 12, 13). The decay is surrounded by a blue-gray border which frequently contains pockets of brown-stained wood. In transverse section it is characterized throughout the heartwood by thin radiating lines of rot which appear to follow the wood rays.

Trametes serialis Fr., associated with a brown cubical trunk rot, was isolated only three times, while the remaining diagnosed infections were referred to miscellaneous fungi which were responsible for single infections.

A large number of the infections, many of them associated with mixed decays, were undetermined. The frequent isolation of a fungus recently identified by Miss Doreen Wells as the imperfect form of *Coryne sarcoides* (Jacq.) Tul. (6), both from decayed wood and from areas in the heartwood unaffected by rot, suggests that this fungus enters the heartwood before decay has been initiated by a recognized decay organism. In many instances, the close association of this fungus with typical decays has prevented the isolation and correct determination of the causal fungus. Although it was possible to assign some of the mixed decays to well known decay fungi on the basis of their gross characteristics, this method was not considered sufficiently reliable in all cases. Nevertheless, many of the undetermined rots are thought to represent various stages of decay caused by fungi that have been reported here, and it is not expected that the relative frequency of the fungi listed in Table I would be seriously altered if positive diagnoses of all these rots had been possible.

Relative Importance of the Fungi Associated with Decay

In Table I are presented the actual volumes of decay attributed to each of the fungi encountered in this study. Though root- and butt-rots were responsible for nearly half of the total number of infections occurring in the original sample, only 30.3% of the total cubic foot volume of decay was attributed to this class of fungi. Calculated on a board foot basis, the basal rots were responsible for a total loss of 2628 board feet, or 35.2% of the total cull due to decay. The average column of basal rot extended 6.6 ft. above the 1 ft. stump section; in some trees, the basal rot column extended up the tree approximately 20 ft. causing such trees to be classed as "total cull". The real importance of basal rots, however, lies not in the actual volume of

decayed wood, although this may be excessive in some trees, but in the structural weakening by such rots which often results in appreciable losses of infected trees through windfall.

Polyporus circinatus var. *dualis*, the most important cause of butt decay, was responsible for over half of the volume losses in the basal portion of the trees and 16.5% of the total decay losses. Five merchantable spruce trees infected by this fungus were regarded as "total cull". The remaining butt rots of importance were *Flammula connissans* causing 6.4% of the total decay losses, "Unknown C" with 3.1% and causing two trees to be culled, and *Coniophora puteana* with 2.0% and causing one tree to be culled.

Among the trunk rots the loss in volume of 34.3% caused by *Fomes pini* exceeded the decay volume for any other fungus and was greater than the combined volume losses resulting from butt rots. On a board foot basis, *F. pini* caused 25.4% of the total volume of cull including the total loss of six merchantable trees. *Stereum sanguinolentum*, the most common fungus encountered, was responsible for only 14% of the total decay losses. Three trees were classed as "total cull" because of infection by *S. sanguinolentum*. *Peniophora septentrionalis* with 6%, and *Trametes serialis* with 2.1%, were the remaining fungi which caused losses exceeding 1.0% of the total decay volume.

The analysis clearly demonstrated the importance of the fungi causing white rots in living subalpine spruce since 93.6% of the total volume losses were referred to this class of fungi.

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AN EVALUATION OF DIFFERENT IONIZING RADIATIONS FOR POSSIBLE USE IN THE GENETIC TRANSFER OF BUNT RESISTANCE FROM AGROPYRON TO WHEAT[1]

By E. N. Larter[2] and F. C. Elliott[3]

Abstract

Ionizing irradiations were employed with the ultimate objective of transferring genes for bunt resistance (*Tilletia* spp.) from a 56-chromosome wheat–*Agropyron* derivative to a hexaploid winter wheat, *Triticum compactum* var. Elgin ($2n = 42$). Various sources of irradiation, including X-rays, thermal neutrons, and radioisotopes (P^{32} and S^{35}) were administered to hybrid seeds. The relative "efficiencies" with which these various sources induced chromosome breakage and subsequent translocation were measured by scoring the frequency of multivalents observed in microsporocytes of treated and control F_1 hybrid plants. Increments of X-radiation caused a decrease in survival and fertility, as well as in chromosome interchange frequency. Thermal neutrons, on the other hand, caused correspondingly increased interchange frequency with increased dosage. Percentage survival of neutron-treated plants, however, was equal to that of controls. Although percentage survival of hybrid plants treated with the two radioisotopes was low, cytological analyses of P.M.C.'s of surviving plants indicated that the number of translocations induced by these sources remained equal to that of control material.

Introduction

The genus *Agropyron* has long been recognized as a possible source of certain valuable traits in wheat–*Agropyron* breeding programs. Winter hardiness, perennial habit, and resistance to drought and disease are but a few such *Agropyron* characters sought by the plant breeder. The transfer and ultimate stabilization of such desired characters in a commercial wheat background, however, have afforded certain difficulties. It is known, for example, that in hybrids involving these two genera there often occurs a complete lack of association between certain of the *Agropyron* chromosomes and those of *Triticum vulgare*. Free chromosomal recombination as it occurs under conditions of intraspecific hybridization, therefore, cannot be expected to occur.

That ionizing radiations can induce chromosome breakage and subsequent recombination is now well recognized. Despite the widely known results of the fundamental researches in radiation genetics by Muller (1928 (2)) and Stadler (1930 (6)), or even earlier work concerning the biological effects of rays from radium and X-rays (Gager, 1908 (1); Petry, 1922 (4); Stein, 1922 (7)), North American plant breeders until recently have made relatively little application of ionizing radiations as a possible source of variability in their breeding programs. Although little is known of the nature of the gene

[1]*Manuscript received in original form October 26, 1955, and as revised, May 2, 1956. Contribution (Scientific paper No. 1442) from Washington Agricultural Experimental Station, Pullman, Wash. Project 966. Based on part of a thesis submitted by the senior author to the State College of Washington in partial fulfillment of requirements for a Ph.D. degree.*

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or of mutations, the fact that discontinuous variation can be created has made it possible to utilize gene mutations as a tool in plant breeding. Moreover, where there exist barriers to natural chromosomal recombination, it is possible by means of irradiation to induce an exchange of chromosome segments carrying genes for certain desirable agronomic characters. By X-radiation of hybrids of *Aegilops umbellulata* × *Triticum aestivum* Sears (1955 (5)) was recently successful in inducing transfer of the *Aegilops* gene for resistance to leaf rust (*Puccinia triticana*) to the wheat parent.

A radiation program designed to ultimately transfer genes governing resistance to bunt (*Tilletia* sp.) from *Agropyron elongatum* (Host) Beauv. to *Triticum compactum* var. Elgin was initiated at the Washington Agricultural Experimental Station. It is not possible at this time to evaluate the success of this particular objective since several generations of plant material will first have to be studied. The purpose of the study reported here, however, was to compare various sources of radiations with respect to their effectiveness in inducing the maximum frequency of chromosomal interchange with minimum lethality of material under study. Information gained from such a study may be of value in future breeding and irradiation programs involving the transfer of desirable genes from *Agropyron elongatum* to our commercial varieties of wheat.

Materials and Methods

The parental material used in these experiments was bunt resistant $2n = 56$ wheat–*Agropyron* derivatives which were originally received as F_1 hybrids from W. J. Sando, Beltsville, Maryland. The parental lines were inoculated each year with a composite of bunt races which identified the *Agropyron* source of resistance. By the F_6 or F_7 generation, these $2n = 56$ derivatives (hereafter referred to as S.H.-191 lines) appeared morphologically uniform under field conditions and relatively stable in their expression of resistance to bunt. A cytological analysis of plants within this population revealed that regular pairing occurred between the wheat and *Agropyron* chromosomes. Twenty-eight bivalents were consistently observed at metaphase I.

During the growing seasons of 1952–54, several hundred spikes of S.H.-191 plants (Fig. 3) were pollinated with pollen from *T. compactum* var. Elgin ($2n = 42$), a winter wheat variety that is susceptible to all known races of bunt. Over 1000 hybrid seeds were obtained from the two-year crossing program. Dormant seeds were subjected to X-ray and thermal neutron radiation, while the radioisotope treatment was applied to young seedlings. The following radiation dosages were employed:

(1) X-radiation administered to 505 dormant seeds at a rate of 550 *r* per minute. Total dosages applied were 10, 12, 14, and 16 thousand *r* units.

(2) Thermal neutrons applied to 300 dormant seeds at a flux of approximately 7.5×10^8 $N_{th}/cm.^2/sec.$ for periods of one, three, and five hours respectively.

(3) Aqueous solutions of radioactive phosphorus at concentrations of 0.2, 2.0, and 8.0 μc. per seed applied to a total of 90 hybrid seedlings.

(4) Aqueous solutions of radioactive sulphur at concentrations of 0.4, 4.0, and 16.0 μc. per seed administered to a total of 90 hybrid seedlings.

In the use of the radioisotopes, the solutions were contained in petri-halves in which the roots of the seedlings were immersed for a period of six days. Transplantations were then made either to greenhouse pots or directly into the field. All irradiated seed was sown immediately after treatment.

At reduction division, pollen mother cells of the treated and control hybrid plants were examined for chromosome associations. Multivalents of three and four chromosomes were considered evidence of one interchange, associations of five and six as two interchanges, and seven and eight as three interchanges. The frequency of multivalents observed at metaphase I of treated and control plants of each treatment was used as an index of the relative efficiency with which the various radiations induced chromosomal interchanges.

Experimental Results

Treated and Control F_1 Hybrids

Survival and Fertility

A summary of the survival and fertility percentages of control (untreated) and treated hybrids for each of the various sources of irradiation used in the present study is shown in Table I.

TABLE I

SUMMARY OF SURVIVAL AND FERTILITY PERCENTAGES OF TREATED AND CONTROL F_1 HYBRIDS OF S.H.-191 × *T. compactum*

Treatment	Hybrid seeds irradiated (no.)	Survival* (%)	Fertility† (%)
Control (untreated)	200	76.0	47.7
X-ray alone			
10,000 *r*	135	73.3	26.5
12,000 *r*	135	67.5	29.0
14,000 *r*	135	48.5	18.4
16,000 *r*	100	38.0	13.4
$N_{th}/cm.^2$			
2.7×10^{12}	100	74.5	44.7
8.1×10^{12}	100	85.5	31.1
13.5×10^{12}	100	74.5	19.4
Phosphorus-32‡			
0.2 μc./seed	30	31.4	12.6
2.0 μc./seed	30	13.3	10.5
8.0 μc./seed	30	00.0	—
Sulphur-35‡			
0.4 μc./seed	30	43.3	18.9
4.0 μc./seed	30	26.7	10.5
16.0 μc./seed	30	13.3	10.3

**Survival data taken at maturity.*
†Fertility data based upon seed setting in primary and secondary florets of each spike.
‡Results from one year's experimentation only.

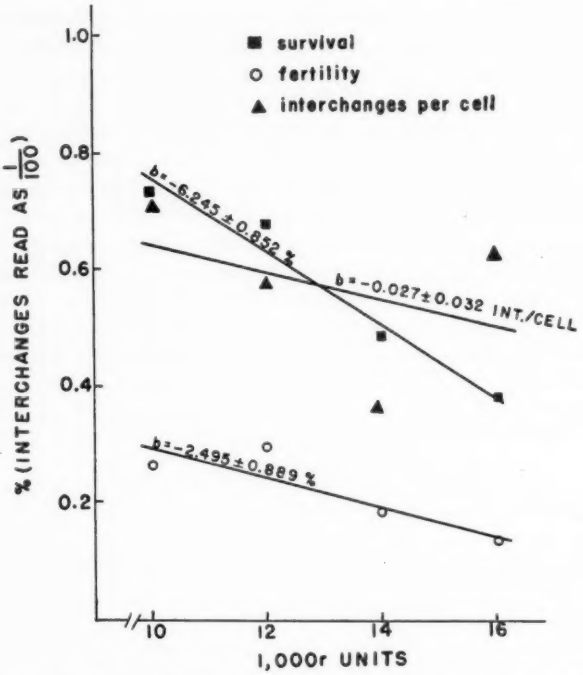

FIG. 1. Linear regression of survival, chromosome interchanges per cell, and fertility on dose of X-rays applied to S.H.-191 × *T. compactum* dormant hybrid seeds.

For the irradiated classes in general, survival and fertility were highest following treatment with neutrons, and lowest following irradiation by the two radioisotopes. With the exception of thermal neutrons, increments in dosage over the range used resulted in a corresponding decrease in both percentage survival and seed set. These various relationships are shown graphically as linear regression lines in Figs. 1 and 2.

Cytology

The analyses of pairing relationships between the *Agropyron* and *T. compactum* chromosome complexes are shown in Table II.

The hybridization of the 56-chromosome derivative and *T. compactum* ($2n = 42$) gave rise to progeny with a mean chromosome number slightly below the expected 49. This discrepancy was not surprising, however, in view of the variation in chromosome numbers found in cells from the same anther as well as from cells from different anthers of the same hybrid plant. Pairing relationship between the wheat and *Agropyron* chromosomes was generally found to be complete as indicated by the mean number of bivalents and univalents in control material approaching the theoretical 21_{II} and 7_{I}.

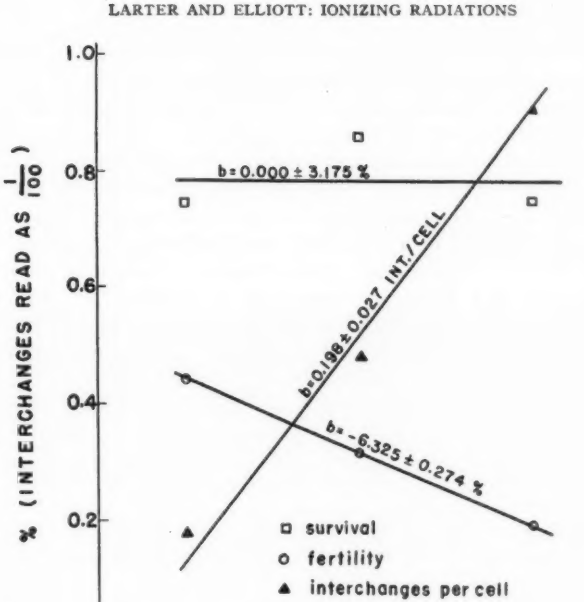

FIG. 2. Linear regression of survival, chromosome interchanges per cell, and fertility on dose of thermal neutrons applied to S.H.-191 × *T. compactum* dormant hybrid seeds.

Lack of complete homology between the two complexes, however, was apparent from the range of univalent frequency appearing in untreated hybrids. Such variation existed also in irradiated classes with apparent loss of chromosomal material as suggested by the low mean of chromosome number per cell in the neutron and S^{35}-treated hybrids.

TABLE II

MEANS AND RANGES OF BIVALENTS, UNIVALENTS, AND CHROMOSOME NUMBERS IN CONTROL AND F_1 POLLEN MOTHER CELLS OF S.H.-191 × *T. compactum* HYBRIDS

Treatment	Cells scored	Bivalents/cell (no.)		Univalents/cell (no.)		Chromosome no.*, mean/cell
		Mean	Range	Mean	Range	
Control (untreated)	202	20.1	15–22	7.9	5–15	48.4
X-ray†	307	18.2	10–22	8.1	5–17	48.0
P^{32}†	82	19.9	13–21	8.3	5–22	48.3
S^{35}†	208	19.8	16–22	6.7	2–12	47.2
Thermal neutrons‡	78	15.7	12–19	8.3	5–15	47.3

**Multivalent associations included.*
†*Subtreatments bulked within each main treatment.*
‡*Only one dose used in 1953 (13.5 × 10^{13} $N_{th}/cm.^2$).*

Regression of chromosomal exchange frequencies on dosage of X-rays and thermal neutrons is graphically illustrated in Figs. 1 and 2.

Results from the thermal neutron-treated population indicated that no relationship existed between interchange frequency and X_1 plant survival. Although the frequency of multivalent associations increased in accordance with dosage increments employed, percentage plant survival remained about the same. This effect was in contrast to that obtained by X-ray. Increments of X-ray dosage resulted in a decreased plant survival as well as a lower frequency of detectable chromosome interchanges. From the standpoint of using X-radiations as a method of induction of chromosome translocation, therefore, the latter observation may be of considerable practical significance.

Because of the low survival percentage of hybrid plants treated with P^{32} and S^{35}, it was not possible to obtain an unbiased estimate of the frequency of translocation induced by these sources. In the surviving hybrids, however, the frequency of interchanges was no higher than that in control material.

Photomicrographs of P.M.C.'s of control and treated hybrids are presented in Fig. 4.

Discussion

In the case of interspecific and intergeneric hybrids, crossing over may be seriously restricted owing to the limited chromosomal pairing. Factors governing economically desirable characters may, as a result, be difficult to transfer to commercially useful gene complexes. The transfer of chromosomal segments by artificial agents such as ionizing radiations, therefore, may have an important place in the plant breeder's program.

The present study was initiated with the ultimate goal of establishing bunt resistance in a high yielding commercial wheat from an *Agropyron*–wheat derivative. It is too early to evaluate accurately the possibility of achieving this end. As suggested by Osborne and Elliott (1955 (3)), early generations of such material should be appraised very rigidly for success attained in the induction of desired chromosomal exchanges. Several plant generations may be required to effect exclusion of all unsynapsed *Agropyron* chromosomal material from the segregating populations.

From a practical point of view, the expenditure of time and labor in obtaining sufficient numbers of hybrid seeds for irradiation purposes, as well as in the handling of subsequent segregating generations, warrants special consideration of the use of the most "efficient" radiation dosages. On the basis of the translocation–survival index as used in the present investigation, it appeared that the thermal neutron treatment was most practical. With increased increments of radiation from this source, an increased chromosome exchange frequency occurred while percentage survival of treated plants remained equal to that of controls.

In contrast with thermal neutrons, however, even the lowest dosage of radioisotopes caused a retarded development of treated plants for a considerable time after treatment. Chromosome interchange frequency in microsporocytes of treated plants was also low for all dosage levels. It is probable,

PLATE I

FIG. 3. Spikes and spikelets of parental and hybrid plants involved in the present study. A. *Agropyron elongatum* (Host) Beauv. ($2n = 70$). B. *T. compactum* Host var. Elgin ($2n = 42$). C. A 49-chromosome hybrid of B × D. D. S.H.-191 ($2n = 50$).

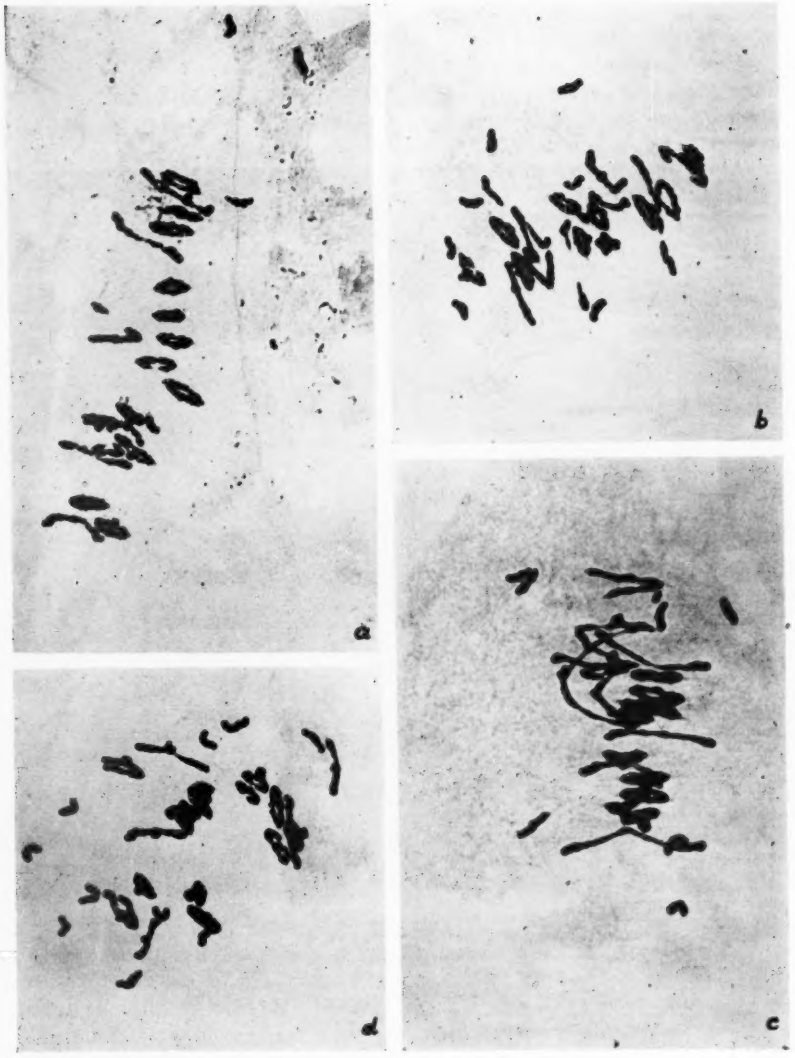

FIG. 4. Microsporocytes from S.H.-191 × *T. compactum* plants.

(*a*) $20_{II} + 3_{I} + 1_{III}$
(*b*) $11_{II} + 8_{I} + 2_{IV} + 1_{VIII}$
(*c*) $13_{II} + 7_{I} + 2_{III} + 1_{VIII}$
(*d*) $17_{II} + 10_{I} + 2_{III}$

therefore, that because of a physiological unbalance created within radioisotope-treated plants, cell division was seriously retarded. The decreased number of chromosome translocations obtained with the higher X-ray dosage as compared with the lower levels may also be attributable to selective elimination of cells that are both physiologically and genetically affected. As a result, a large proportion of those plants surviving the higher doses of X-ray revealed the least chromosomal disturbance.

From the standpoint of the practical usage of radioisotopes, it is probable that they were applied in the present study after tiller differentiation had occurred. Several tiller primordia arise quite early in the embryonic stage of development in cereals. It is apparent, therefore, that any chromosomal structural changes induced by treatment in the seedling stage of growth would have little chance of being found in a large percentage of germ cells. Injection of dilute concentrations of the radioisotope into hybrid plants either shortly before microsporogenesis or after fertilization may be a more effective means of treatment for maximum practical results.

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